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Tinnitus: from cortex to cochlea

Geven, Leontien

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Tinnitus

From cortex to cochlea

Leontien Ingeborg Geven

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Tinnitus

From cortex to cochlea

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Contents

Chapter 1

Introduction to the thesis

1.1 Tinnitus

The word tinnitus comes from the Latin word "tinnire", which means to ring. Tinnitus is the perception of a meaningless sound without an external source. It is described by patients in many ways, for example as a ringing, hissing or buzzing sound. Tinnitus can be lateralized to the left or right ear, or be perceived in the middle of the head. There are two types of tinnitus: objective and subjective tinnitus.

Objective tinnitus is a sound that can be perceived by not only the patient, but also by an external observer. Objective tinnitus can be of vascular or muscular origin. Examples are a stenosis of the carotic artery, a sinodural fistula, a paraganglioma or a myoclonic contraction of the soft palate or the stapedial muscle (Chandler, 1983; Howsam et al., 2005; Sonmez et al., 2007). These conditions can give rise to a noticeable, pulsating sound, sometimes in synchrony with the heartbeat. The sound gives its auditory percept through the normal hearing mechanism, starting with perception in the cochlea. Because objective tinnitus has an identifiable source, treatment options are sometimes available, depending on the specific source. Objective tinnitus is rare, but because of potential treatment options or medical consequences, it needs to be assessed when a patient with tinnitus seeks medical attention.

Subjective tinnitus cannot be heard by an external observer, but only by the patient. In this case there is no identifiable sound source, making subjective tinnitus a phantom percept. Subjective tinnitus is the most frequent form of tinnitus, and the subject of this thesis. Therefore we will not use the adjective "subjective" throughout this thesis anymore.

1.1.1 Prevalence

Transient tinnitus is experienced by almost all adults at some point in their life. Permanent tinnitus is also a very frequently reported symptom, with prevalence ranging from 8-20% in the general population (Baguley et al., 2013). Also, 1-3% of the patients are severely affected by it, with a marked reduction in quality of life (Langguth et al., 2013). Tinnitus can even bring people to suicidal thoughts. Tinnitus is more common in men than women and increases with age. For men between 65 and 74 years of age, tinnitus prevalence is almost 12% (Axelsson and Ringdahl, 1989; Lockwood et al., 2002; Roberts et al., 2010). It is also frequently reported as a disability in people returning from combat situations (Lew et al., 2007). With the increasing risk of hearing loss with exposure to

for example loud music, tinnitus is a major current and future health problem (Roberts et al., 2010).

1.1.2 Pathophysiology of tinnitus: current concepts

Dissecting the vestibulocochlear nerve as a treatment for tinnitus was not effective in approximately half of the cases (reviewed by Kaltenbach, 2006). Moreover, cutting the vestibulocochlear nerve for removal of acoustic tumours led to tinnitus in 50% of the subjects that did not experience tinnitus before the surgery (Berliner et al., 1992). This contributed to the hypotheses that tinnitus is a central, rather than a peripheral auditory phenomenon. The hypotheses on the pathophysiology of tinnitus generally concern neuroplastic changes in the central auditory system, probably initiated by some form of cochlear damage (Eggermont and Roberts, 2004; Møller, 2006; Roberts et al., 2010; Kaltenbach, 2011; Norena and Farley, 2013). The precise mechanisms behind tinnitus are not disentangled yet. For example, it is not clear why not everybody with hearing loss develops tinnitus.

The location and the form of neuroplastic changes that would lead to tinnitus have been the subject of an increasing number of studies. A disturbance in the balance between excitation and inhibition after cochlear damage is believed to result in tinnitus, but the exact nature of this disturbance is subject of many scientific investigations. Increases in spontaneous neural activity are demonstrated at several levels of the auditory system after peripheral damage (Norena and Eggermont, 2003; Kaltenbach, 2006; Mulders and Robertson, 2009; Vogler et al., 2011). Increased activity in the left primary auditory cortex in human tinnitus patients is also demonstrated with functional imaging (Arnold et al., 1996; Langguth et al., 2006). Recently, increased synchrony in spontaneous activity is hypothesized to be involved in the perception of tinnitus (Norena and Farley, 2013). Possible, the efferent auditory system is also involved in the disbalance between excitation and inhibition, but is currently mostly overlooked (Jastreboff, 1990; Bauer, 2004; Kaltenbach, 2011).

1.1.3 Current treatment options

With its high prevalence and potentially devastating consequences, tinnitus patients are in urgent need of a cure. Unfortunately, there is no definite cure for all patients. Counselling with non-specific support offers some relieve to the tinnitus disturbance (Dobie, 1999), with potential greater effect if specialised cognitive behavioural therapy is applied (Cima et al., 2012). Tinnitus can be managed to some level with the use of hearing aids or sound generators (e.g. Parazzini et al., 2011; Shekhawat et al., 2013).

Several types of medication have been proven ineffective in suppressing tinnitus, for example anticonvulsants (Hoekstra et al., 2011) or Ginkgo biloba (Hilton et al., 2013). More invasive types of therapy are subject of interest as well. Electrodes placed around the vestibulocochlear nerve showed a reduction in tinnitus burden (Bartels et al., 2007), as well as extradural electrodes on the auditory cortex in selected patient groups (De Ridder et al., 2011). Also, cochlear implants are considered as a treatment option for single-sided deaf patients with tinnitus (Arts et al., 2012). Recently, repetitive transcranial magnetic stimulation has been used to as a non-invasive treatment option for tinnitus (Ridding and Rothwell, 2007). But a recent review showed limited support for long-term effect (Meng et al., 2011). With the high numbers in prevalence and reduction in quality of life caused by tinnitus, a curative therapy for all tinnitus patients would be ever so welcome.

1.2 Central auditory pathway

This section briefly describes the central auditory system. As mechanisms in the central auditory system appear to play a crucial role in tinnitus, the anatomy of the auditory portions of the brain is relevant for interpreting the following chapters.

1.2.1 Afferent central auditory pathway

Sound enters the central auditory system through the cochlea. In the cochlea the sound waves are transformed into electric activity and sent through the auditory nerve (n VIII). The auditory nerve fibers connect to the ipsilateral cochlear nucleus, in the brain stem. The cochlear nucleus itself can be divided in a dorsal part, and an anterior and posterior ventral part, based on location and cytoarchitectural differences. The tonotopic representation of the perceived sound is kept intact in each of the three divisions of the cochlear nucleus.

After the cochlear nucleus, the auditory pathway continues to the superior olivocochlear complex (SOC), located in the pons. It receives fibers from the ipsilateral as well as the contralateral cochlear nucleus. This makes the SOC the first stage for binaural processing of sound. Each SOC can again be divided in a lateral nucleus and a medial nucleus. The medial olivary nucleus receives the neurons for the binaural sound processing.

Next, the pathway connects to the inferior colliculus (IC) in the midbrain. Part of the IC receives direct input from the cochlear nucleus through the lateral lemniscus. The IC can be divided in three parts: the central, the external and the pericentral nucleus.

Tonotopic organisation is held intact through the central part of the IC. The external portion of the IC receives multimodal information. For example, there are connections to motor neurons that control head or eye movements in response to sound. Therefore, the IC is able to recognise the location of the sound source.
The last nucleus before the auditory cortex is the medial geniculate body (MGB) in the thalamus. This is regarded as a relay station for signals coming from the cochlea to the auditory cortex. The ventral part is tonotopically organised. The dorsal part of the MGB is not tonotopically organised and is believed to be involved in processing complex sounds. The medial part of the MGB receives input from non-auditory sensory brain areas, making responding to more than one sensory modality possible. These different parts of the MGB connect to specific parts in the auditory cortex, which is the next and final part of the central auditory pathway.
The auditory cortex is located on both temporal lobes, more specifically on the superior part. The lateral fissure named Heschl's gyrus holds the primary auditory cortex (PAC). The PAC, also known as Brodmann area (BA) 41 is tonotopically organised (Langers and van Dijk, 2012). Based on cytoarchitectural differences, other areas have been identified along the temporal lobe, with the secondary auditory cortex (BA 42) and the auditory association cortex (BA 22) as the most relevant for this thesis.

1.2.2 Efferent central auditory pathway

In contrast to the ascending auditory pathway, the descending (or efferent) auditory pathway is much less known. Rasmussen was the first to describe a part of the efferent system in 1953 (Rasmussen, 1953). Descending nerve fibers from all the different parts of the central auditory pathway connect to every level, including the cochlear nucleus and cochlea. It is involved in multimodal processing and learning, with interactions from emotions, language, and attention. Currently, it is believed that the efferent system controls feedback, motor and relay functions in the central auditory system. In an extensive review by Schofield the extensiveness of the efferent system is described (Schofield, 2011). A plethora of connections in loops, chains and branches are described that are involved in the efferent processing of sound. These extensive connections suggest a major role of top-down influences in auditory processing and perception.

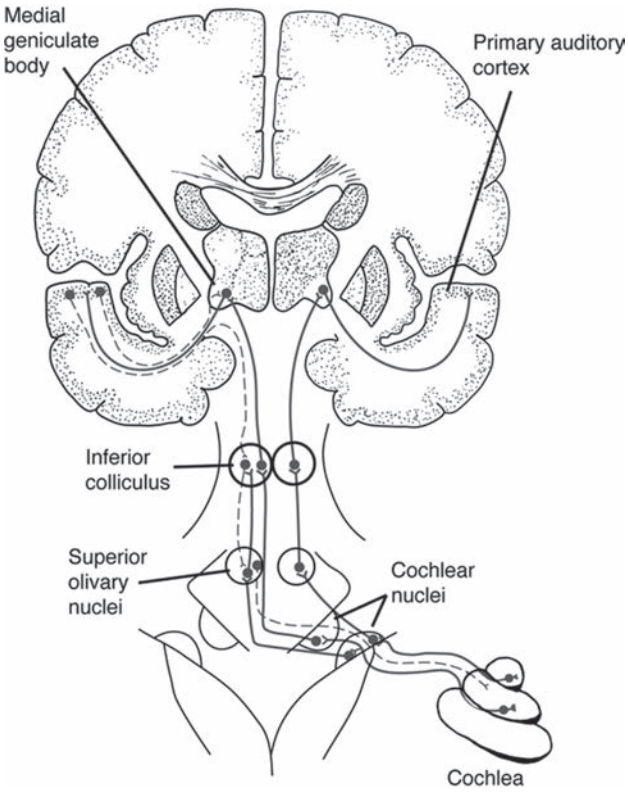

Figure 1. Schematic overview of the central auditory pathway, with afferent (solid line) and efferent components (dashed line) (reprinted with kind permission from K. Boyen, 2013)

1.3 Objective and outline of this thesis

1.3.1 Objective

Since the efferent system plays a major function in auditory processing, it may also be involved in the mechanisms that cause tinnitus. This thesis aims to obtain more insight into the origin of tinnitus with special emphasise on the efferent auditory system. To do so, different techniques have been used to explore various part of the central auditory system.

1.3.2 Outline

The thesis is outlined from the cortex to the cochlea.

In **Chapter 1** –the current chapter– a general introduction to the central auditory system and the current knowledge on tinnitus is provided.

The potential role of the efferent auditory system in tinnitus is currently mostly overlooked. In **Chapter 2** the central efferent auditory system is reviewed. Most of our knowledge is based on animal research, although some experiments that are described have studied the efferent auditory system in humans. The goal of this chapter is to describe the available knowledge and try to answer the question of the specific role for the efferent auditory system in tinnitus.

The primary auditory cortex can be a starting point in trying to objectify a phantom auditory sensation. Also, it may be viewed as the starting point of the efferent auditory system. **Chapter 3** is an attempt to provide an objective measure of tinnitus, using Positron Emission Tomography (PET) to visualize increased brain metabolism. Previous reports pointed to an increased metabolism in the left primary auditory cortex in tinnitus patients, but this was not always tested in control subjects. This unilateral hyperactivity has been used as a target in localized treatments such as Transcranial Magnetic Stimulation (TMS). But this type of stimulation has only a modest effect. Therefore, we set out to study resting-state metabolic activity by FDG-PET in subjects with bilateral tinnitus, and to compare their results to those of control subjects without tinnitus. In our analysis, we specifically focused on asymmetries between the left and right auditory cortices in order to allow for a straightforward comparison to earlier studies.

The part of the efferent auditory system in humans that can be tested non-invasively, is the medial olivocochlear efferent system. With the use of contralateral sound, the efferent influences on otoacoustic emissions can be demonstrated. In **Chapter 4** the functioning of the medial olivocochlear efferent system in tinnitus patients is compared to healthy control subjects.

In the study described in **Chapter 4**, results were contradictory to previously published results. Therefore, we felt the need for a supplementary analysis on the contralateral suppression of otoacoustic emissions. Since otoacoustic emissions do not only include

frequency information, but also temporal information, we used wavelet analysis in **Chapter 5** to test for differences between patients and controls**.**

If the efferent auditory system is dysfunctional in tinnitus, testing it non-invasively in humans will be a necessary technique to detect this. In **Chapter 6** we have tried to influence the efferent auditory system with TMS of the auditory cortex. TMS can stimulate brain regions through an intact skull and scalp. It induces temporary changes in brain activity that outlast the stimulation itself. With the measurement of OAEs we tried to detect the efferent influence on the OAE amplitude.

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Chapter 2

Plasticity in tinnitus patients: a role for the efferent auditory system?

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2.1 Introduction

Tinnitus is a phantom perception of a meaningless sound in the absence of an external source. It is a very frequently heard complaint. It is estimated that 14% of the population suffers from chronic tinnitus, and 2% is severely affected by it (Axelsson and Ringdahl, 1989; Lockwood et al., 2002). Its prevalence increases with age, to the level of 20 % in people over 50 years of age, depending on the definition used to describe tinnitus (Hoffman and Reed, 2004). Tinnitus is associated with hearing loss and cochlear damage. Dissecting the vestibulocochlear nerve as a treatment for tinnitus is not effective in approximately half of the cases (reviewed by Kaltenbach, 2006). Moreover, cutting the vestibulocochlear nerve for removal of acoustic tumours led to tinnitus in 50% of the subjects that did not experience tinnitus before the surgery (Berliner et al., 1992). This contributed to the hypotheses that tinnitus is a central, rather than a peripheral auditory phenomenon. These hypotheses on the pathophysiology of tinnitus generally concern neuroplastic changes in the central auditory system, probably initiated by some form of cochlear damage (Eggermont and Roberts, 2004; Moller, 2007; Roberts et al., 2010; Kaltenbach, 2011; Norena and Farley, 2013).

Currently, the theory concerning central gain is of interest. With a reduced peripheral input in the case of a hearing loss, the central auditory system is believed to strive for a homeostatic balance by upregulating the sensitivity of the central auditory neurons. Tinnitus is then a byproduct of this central gain, for example due to increased spontaneous firing rate or changes in temporal coherence. Noreña and Farley proposed that both the balance in central gain after peripheral damage, and residual peripheral spontaneous activity leads to perceiving tinnitus (Norena and Farley, 2013). Many different locations within the central auditory system show changes in spontaneous firing rate after peripheral damage (Norena and Eggermont, 2003; Kaltenbach, 2006; Mulders and Robertson, 2009; Vogler et al., 2011). Thus changes in the balance between excitation and inhibition are likely to play a role and therefore it seems logical that auditory plasticity in response to the cochlear damage can be a key factor.

Several agents are related to tinnitus in humans, such as noise trauma, age-related hearing loss or ototoxic medication, suggesting different causal mechanisms also. Consistent with that, Tyler et al. (2008) reported preliminary results of a cluster analysis based on the clinical data of 246 tinnitus patients, identifying 4 subgroups with different characteristics. Noise trauma is the variety that has been studied the most in animal

experiments for tinnitus. Usually, the noise trauma generates a high frequency hearing loss, which resembles the hearing loss of the general tinnitus patient. Increases in spontaneous firing rate have been demonstrated at different levels in the central auditory system, for example the dorsal cochlear nucleus (Kaltenbach, 2006), inferior colliculus (Mulders and Robertson, 2009), or the auditory cortex (Norena and Eggermont, 2003). But not all people with high frequency hearing loss experience tinnitus and it remains unclear why. Potentially, there are multiple mechanisms that lead to some form of central neuroplastic changes, which can result in tinnitus.

As in every other efferent sensory system, the efferent auditory system, as a top-down control mechanism, is thought to participate in regulation and feedback of activity in the central auditory system. Abnormal functioning of this system could therefore contribute to the plasticity involved in tinnitus, but this is currently mostly overlooked (Jastreboff, 1990; Bauer, 2004; Kaltenbach, 2011). In humans only the medial olivocochlear (MOC) efferent system, a small part of the entire efferent auditory system, is routinely probed for its role in contralateral suppression of otoacoustic emissions (Guinan, 2006). The MOC controls activity in the cochlea through efferent projection to the outer hair cells. The MOC is hypothesized to aid in better detection of signals in noisy environments, to provide protection against acoustic trauma and to be involved in selective attention when confronted with multiple stimuli (for review Guinan, 2006; Palmer et al., 2007; Pickles, 2008). When presenting a contralateral sound, the MOC reflex influences the outer hair cells and modulates the amplitude of the otoacoustic emissions generated by them, usually by reduction of the amplitude (for review see Guinan, 2006). Thus the MOC reflex can be tested non-invasively and is therefore highly suited as an application in human research.

Several studies have been conducted to test for dysfunction of the MOC reflex in humans with tinnitus (Chery-Croze et al., 1993; Chery-Croze et al., 1994a; Chery-Croze et al., 1994b; Attias et al., 1996; Lind, 1996; Ceranic et al., 1998; Favero et al., 2006; Riga et al., 2007; Granjeiro et al., 2008; Fernandes Lda and Santos, 2009; Geven et al., 2011; Paglialonga et al., 2011; Geven et al., 2012). The amount of reduction of the otoacoustic emission with contralateral acoustic stimulation (CAS) was measured for tinnitus patients and in some studies compared to healthy control subjects. Measuring inhibition caused by the MOC effect has technical challenges, as reviewed by Guinan (2006; 2010). In several studies the contralateral ear of the tinnitus patient was used as a control measure (Chery-Croze et al., 1993; Chery-Croze et al., 1994a; Chery-Croze et

al., 1994b; Lind, 1996; Riga et al., 2007), which can be disputed, as tinnitus appears to be a central phenomenon. Overall, a clear difference in the amount of OAE reduction was not demonstrated. However, although dysfunction of the MOC reflex was not demonstrated by differences in contralateral suppression of OAEs, it does not mean that the MOC efferent system is not involved in tinnitus, as we will describe in more detail later.

While the (dys)functional status of the MOC efferent system has thus been investigated in tinnitus patients, the more central part of the efferent auditory system in humans still remains open for investigation for its role in tinnitus. Because the efferent system might play a role in the neuroplastic changes after cochlear damage, the goal of this paper was to investigate the current knowledge of the functional efferent auditory system in humans, mostly based on animal research, and to look for new possibilities to try to determine the role of the corticofugal efferent auditory system in human tinnitus.

2.2 The efferent auditory system in animals

2.2.1 The anatomy

Rasmussen was the first to describe the olivocochlear bundle as an efferent system (Rasmussen, 1953). In a very recent review by Schofield the current knowledge about the anatomical connections at all the different levels of the auditory efferent system were described (Schofield, 2011). Also, the evolution of the efferent system across vertebrates was discussed by Köppl (2011). In summary, projections are abundant at all levels and appear to form multiple functional feedback loops as well as downstream, sometimes parallel running, projection chains (Fig 1). The corticofugal projections connect to all levels of the central auditory pathway, some in parallel with projections from lower levels connecting with the cochlear nucleus. In addition, the auditory efferent system sends fibers to non-auditory areas such as the amygdala (Pickles, 2008). For the tonotopically-organised areas the tonotopic organisation seems to remain intact in their projecting fibers, for example for corticothalamic (Schofield, 2011) as well as cortico-collicular connections (Bajo et al., 2007; Lim and Anderson, 2007). In summary, there exists a rich morphological substrate for auditory feedback loops and modulation of downstream responses at every level of the auditory pathway.

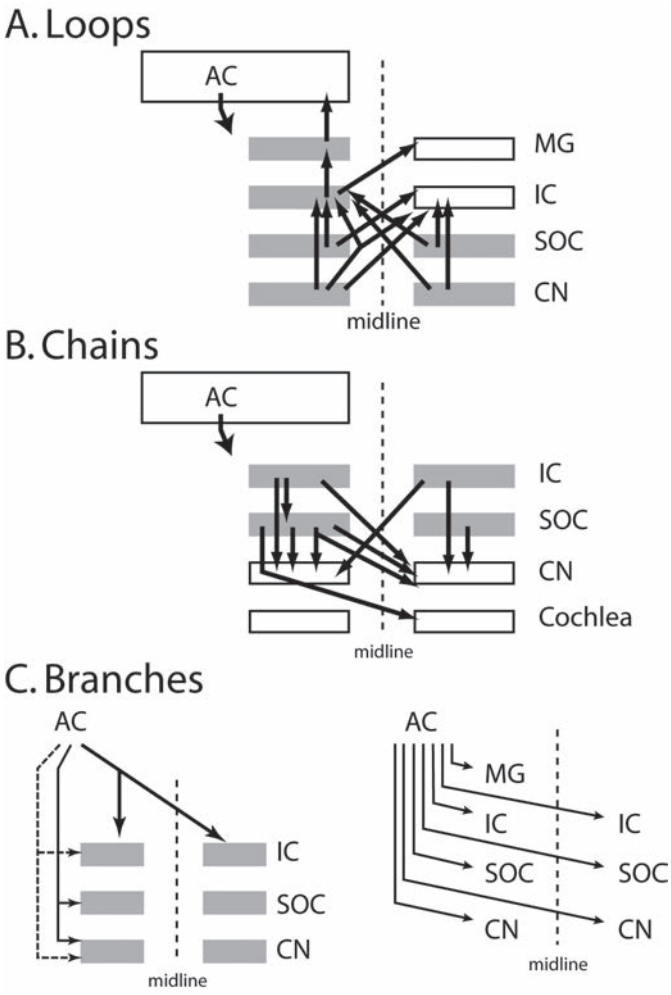

Fig 1. Illustration of the plethora of descending pathways of the auditory system, both ipsi- and contralateral. Cortical projections can target all other levels in the auditory system, which give rise to ascending (a) and descending pathways (b) (gray boxes are the starting point of the arrows). Current knowledge is expanding with demonstration of loops and chains in the auditory system (a,b). With branching of descending axons activity is thought to be coordinated at different levels within the auditory system (c) (reprinted with kind permission from Springer Science+Business Media and Schofield: his figure 9.10)(Schofield, 2011).

2.2.2 The function of the efferent auditory system in animals

In his extensive review of corticofugal modulation, Suga et al. describe the various changes that can occur during electrical or chemical stimulation or ablation of various regions of the efferent auditory system (Suga et al., 2011). Mostly the changes shown were shifts in "best frequency". With electrical stimulation of cortical auditory neurons,

Suga and Ma demonstrated both facilitation and inhibition of auditory responses in lower-level auditory regions (for review see Suga and Ma, 2003). Even down to the level of the cochlea this change was observed in cochlear hair cells after electric stimulation of cortical auditory neurons in bats (Xiao and Suga, 2002). Depending on whether the stimulated cortical neuron was matched to the cochlear frequency measured, the amplitude of the outer hair cell activity increased or decreased. The corticofugal system is thought to mediate these changes. In general, the corticofugal system reorganizes the cortical input, using auditory experience (Suga et al., 2011).

Nakamoto et al. published another example of the function of the efferent system in animals. They tested the role of corticofugal modulation in a situation with multiple, concurrent sound sources (Nakamoto et al., 2010). After localized cooling of the primary auditory cortex in guinea pigs the synchronization of the responses in the inferior colliculus changed, and thus the neural representation of the concurrent stimuli changed, proving the alterations were caused by the corticofugal efferent auditory system. Bajo et al. demonstrated yet another role of the corticofugal system in auditory plasticity (Bajo et al., 2010). Ferrets are normally able to re-learn correct horizontal sound localization after their spatial interaural cues are altered by occluding one ear. However, this re-learning was disrupted by the selective ablation of a major efferent pathway, the corticocollicular connections from layer V neurons of the primary auditory cortex to the inferior colliculus. Interestingly, the same learning impairment was demonstrated after partial ablation of olivocochlear efferents (Irving et al., 2011). It thus appears that an intact efferent chain, from the cortex to the cochlea, is necessary for auditory plasticity in the context of sound localization.

2.2.3 Tinnitus and the afferent and efferent system in animals

After establishing that noise trauma resulted in increased spontaneous firing rate in the inferior colliculus (IC) after peripheral (cochlear) damage (Mulders and Robertson, 2009), behavioural evidence of tinnitus after noise trauma was demonstrated (Robertson et al., 2013). Immediately after the acoustic trauma, the hyperactivity in the IC seems to depend on peripheral afferent input. Indeed, upon complete removal of the cochlea, the hyperactivity in the inferior colliculus was reduced, suggesting that the central hyperactivity in the IC depended on the peripheral input of the cochlea (Mulders and Robertson, 2009). This is consistent with recordings in brain slices taken from mice after noise trauma. As activity in brain slices does not depend on afferent or efferent input, the changes observed in brain slices suggest an initial intrinsic reduction in IC

spontaneous activity (Basta and Ernst, 2005). But when the recovery time between the acoustic trauma and the cochlear ablation was increased to 8 weeks, the hyperactivity in the IC did not change significantly (Mulders and Robertson, 2011). So, it seems that there could be a "window of plasticity" for the afferent auditory system in which the increased spontaneous firing rate of the IC can be manipulated by influencing the cochlea. After this time window, the central effects of the noise trauma cannot be changed with manipulation of the peripheral afferent input.

Perhaps stimulation of the efferent system can play a role in alteration of hyperactivity in the central auditory pathway (Mulders et al., 2010). The MOC efferent system was investigated for its role in changing this increased spontaneous firing rate in the inferior colliculus 2 to 4 weeks after noise trauma. Mulders et al. demonstrated that stimulation of the olivocochlear bundle suppressed the increased spontaneous firing rate (that had occurred after an acoustic trauma) in the inferior colliculus in guinea pigs (Mulders et al., 2010). Interestingly, the strength of the medial olivocochlear activity was demonstrated to play a role in the amount of hearing loss after an acoustic trauma. Maison and Liberman were able to define "tough" versus "tender" ears of guinea pigs based on the strength of the MOC reflex (Maison and Liberman, 2000). They found a strong inverse correlation of r=-0.78 between the MOC reflex strength and the hearing threshold shift after noise exposure. Thus when tinnitus is preceded by increased spontaneous firing rate in the IC after peripheral acoustic trauma as discussed above, then moderating this increase via activation of first the afferents in acute tinnitus and later the MOC efferent system in chronic tinnitus could be worthwhile exploring.

2.3 The corticofugal auditory system in humans

The functional connectivity of the corticofugal systems in humans is of course not easy to explore. Here, we provide some examples of studies that demonstrated the existence of a functional corticofugal auditory system in humans. In an elegant study, Perrot et al. tested the human auditory efferent system from cortex to cochlea by means of intra-cerebral electric stimulation of the auditory cortex. As a surgical preparation for drug-resistant epilepsy, 10 patients underwent intracranial implantation of electrodes. The electrodes could stimulate selectively both auditory areas as well as non-auditory areas. The stimulation of the auditory areas resulted in a significant reduction of the contralateral evoked otoacoustic emission amplitude, whereas stimulation of the non-

auditory areas showed no reduction in otoacoustic emissions. This provided evidence for a functional connection between the auditory cortex and the contralateral outer hair cells in humans. The authors safely assumed that the final link in the chain is the MOC efferent system (Perrot et al., 2006). These findings correspond to some extent to the experiments of Xiao and Suga as described above, in which they electrically stimulated the cortex of bats and recorded the changes in the cochlea (Xiao and Suga, 2002). The results were also consistent with observations in patients after temporal lobe resection including Heschl's gyrus, showing a less functional MOC on the contralateral side of the resection (Khalfa et al., 2001).

In line to some extent with the study method of Perrot et al. (2006), Schönfeldt-Lecuona et al. also tested the functional efferent connectivity, although with non-invasive electrical stimulation (Schonfeldt-Lecuona et al., 2012). Instead of direct electrical stimulation, they used transcranial magnetic stimulation (TMS) on the superior temporal gyrus. TMS uses a rapidly changing magnetic field to induce weak electrical currents in the cortex in close proximity to the TMS coil. Depending on the chosen parameters for the treatment protocol, brain activity in the cortex can either be increased or decreased. TMS can be used to elicit cortical activity in all kinds of brain regions, and it has also been used in trials for the treatment of tinnitus (for example see Plewnia et al., 2003; Kleinjung et al., 2005; Langguth et al., 2006b; Rossi et al., 2007). The rationale for treating tinnitus with TMS is the reported hyperactivity in the left primary auditory cortex in tinnitus patients when measured with functional imaging, such as Positron Emission Tomography (PET) (Arnold et al., 1996; Langguth et al., 2006a, for review see Lanting et al., 2009). With TMS of the auditory cortex, Schönfeldt-Lecuona et al. did not find changes in hearing level thresholds or distortion-product OAEs (DPOAEs) of the tinnitus patients, suggesting that no auditory efferent activity was induced (Schonfeldt-Lecuona et al., 2012). However, the localized magnetic field might have been too small to stimulate the auditory corticofugal system. Also, subjects probably had to remove earplugs they wore during the TMS before the DPOAEs could be measured. This could be a significant time delay between TMS stimulation and the start of the DPOAE measurement such that any suppressive effects might have partly decayed. Thus the lack of an effect of TMS on OAEs does not support the functionality of cortico-cochlear connections; however several confounding factors may have contributed to this result.

2.4 Future perspectives of tinnitus and the efferent system

Because tinnitus is believed to be the result of central auditory plasticity after cochlear damage, and the efferent auditory system could play a role in this as explained above, we suggest several potential avenues of research to gain insights into the efferent system and tinnitus in humans. If the corticofugal system plays a role in tinnitus, then this should be explored and potential research strategies can be thought off, both in humans and animals.

2.4.1 Efferent system and sound localization

Recent animal studies showed that a functional efferent system was necessary for re-learning sound localization after altering external cues (Bajo et al., 2010; Irving et al., 2011), as discussed in section 2.2.2. If the plasticity changes that are causing tinnitus were due to a dysfunctional efferent system, an indirect way to assess this could be to test the learning capacities of tinnitus patients to learn new sound localization cues. Of course, one cannot inflict permanent changes of auditory cues in humans. With an elegant study, Hofman et al. investigated a temporary change in auditory cues in humans (Hofman et al., 1998). They changed the shape of the outer ear (pinna) in 4 adult human subjects with an ear mold. This changes the pinna's sound filtering characteristics that are used to localize sound in the vertical plane. After an initial period with poor localization capacity, the subjects reacquired their localization capacities with their "new" pinna. The original sound localization strategies were preserved, which was demonstrated by good sound localization immediately after removal of the outer ear mold (Hofman et al., 1998). If we assume that the changed auditory input triggers a learning effect in sound localization, the efferent system appears to be necessary to facilitate this learning (as discussed above). If the corticofugal efferent system in tinnitus patients is not working adequately, one could image that this can be demonstrated in differences in their sound localization capacities after fitting an outer ear mold, in comparison to non-tinnitus patients. As multiple studies with contralateral suppression of OAEs did not demonstrate a dysfunctional MOC system, the potential difference could then be attributed to the more central portion of the descending efferent system (i.e. from the cortex to the olivocochlear nucleus).

2.4.2 Enhanced plasticity early in life: Children and tinnitus

It is well known that the potential for neural plasticity is significantly greater in young, still developing brains as compared to adult brains (for example see Bischof, 2007). Because tinnitus is believed to be related to plasticity effects after cochlear damage, the enhanced plasticity of childrens' brains can be of special interest. But tinnitus in children is still a subject receiving little attention. Tinnitus is usually not spontaneously reported by children and also not routinely checked. The prevalence of tinnitus in children is estimated to be 6 to 36%, depending on whether children reported it spontaneously or whether they were asked about it (reviewed by Shetye and Kennedy, 2010). This number is even higher in hearing impaired children (Savastano et al., 2009; Juul et al., 2012). In consulted adolescents, the prevalence of tinnitus is estimated at 31 to 37% (Bulbul et al., 2009).

In children with a cochlear implant, tinnitus prevalence was 38% when directly asked about it (Chadha et al., 2009). The duration of the tinnitus was not always stated. When comparing long-latency auditory evoked potentials (AEPs) in patients (children and adults) before and after cochlear implantation, there seemed to be an ongoing maturation of the auditory cortex after implantation, however, not entirely to the level of normal hearing individuals' AEPs (Eggermont, 2008). The parts of the auditory system that are responsible remain to be identified. Is it the afferent or the corticofugal system or both? The prevalence of tinnitus of around 30% seems to be consistent across age groups. But longitudinal follow-up is needed to see whether the tinnitus is persistent in individual patients as they age. Could it be that with the plasticity in younger age the brain can adapt and therefore resolve tinnitus? Or - a less pleasant possibility – could enhanced plasticity even increase the likelihood of developing and maintaining tinnitus? A long-term follow-up study is needed to see what happens with the tinnitus of the affected children over time. The group of children that might be candidates for a cochlear implant can be of special interest, because of the indication of prolonged maturation of the brain.

Thus although the mechanisms responsible are not clear, a long-term follow-up of tinnitus patients with special emphasis on children might give new insight into the role of plasticity of the brain in tinnitus. Interventions such as a cochlear implant provide unique opportunities to test the plasticity of the auditory system.

2.4.3 Efferent auditory system strength

As tinnitus is associated with cochlear damage, detecting individuals who are at increased risk after noise exposure can be helpful. The efferent auditory system, and specifically the MOC, appears to play a role in protecting individuals from hearing loss after noise exposure, as described in section 2.2.3 based on the study of Maison and Liberman (2000). They suggested that MOC reflex testing in humans could be of help to identify those individuals who are at risk of developing hearing loss after occupational noise exposure. Backus and Guinan tested the MOC reflex strength in normal hearing human subjects (Backus and Guinan, 2007). They defined a normalized MOC reflex strength, by comparing the OAE amplitude changes from the MOC reflex with OAE changes caused by two-tone suppression. When averaging MOC reflex effects on OAEs across several different frequencies in 25 subjects, the normalized MOC reflex strength showed an average amplitude suppression of 35%, and ranged from 15% to 60% (Backus and Guinan, 2007). Thus although small, it is possible to measure MOC reflex strength variation in humans. With this in mind and knowing that tinnitus is associated with hearing loss but not present in all individuals with hearing loss, it would be informative to test whether the strength of the MOC reflex is also associated with the onset of tinnitus. As mentioned in the introduction, a clear difference in the amount of OAE reduction with standard contralateral suppression was not demonstrated. For the next step, it would be interesting to see whether the above mentioned strength of the MOC reflex is different between individuals with tinnitus and those without tinnitus. A caveat is that the suppression of OAEs declines with advancing age (Castor et al., 1994; Kim et al., 2002), whereas tinnitus prevalence increases with age (Lockwood et al., 2002). However, another option is to perform a long-term prospective study. Ideally, one would test a large group of individuals for their MOC reflex strength and hearing level, and after some extended period of time, the incidence of tinnitus and hearing loss in the test group is recorded. If this revealed a strong correlation, the MOC reflex strength could be used as an indicator to know who is at risk for developing tinnitus, as a result of cochlear damage.

With regard to the variability in MOC reflex strength, an interesting phenomenon present in tinnitus patients is residual inhibition. Residual inhibition is the continued reduction or abolition of tinnitus after a masking sound is turned off (Vernon, 1977). It appears that residual inhibition is present up to 75% of the tinnitus patients (Moore, 2012). Its duration is usually in the range of 5 to 45 seconds (Roberts et al., 2006). The sounds needed to produce residual inhibition seem related to the frequency of the

hearing loss and the perceived tinnitus (Roberts et al., 2008). To our knowledge, residual inhibition and the MOC reflex have not been linked to each other. Could it be that the duration of the individual residual inhibition is linked to the activity level or strength of the MOC reflex? It seems that through measuring the MOC reflex strength, a few hypotheses can be tested to examine the role of the olivocochlear efferent system in the susceptibility for perceiving tinnitus.

Perhaps studies targeting MOC function with MOC reflex and contralateral suppression in tinnitus patients are too limited. With MOC effect research the role of the inner hair cells (IHC) is not tested. Recent research showed deafferentation of IHC after noise trauma, despite only a temporary threshold shift in hearing level, and recovery of outer hair cell function (when measured by distortion product OAEs)(Kujawa and Liberman, 2009). Such deafferentation of the IHC has recently been linked to tinnitus. Singer et al. have demonstrated that IHC ribbon loss (as a measure for deafferentation) is related to tinnitus when the afferent auditory brainstem response did not completely restore after noise trauma and the protein Arc (an activity-regulated cytoskeletal protein) was not increased in the central auditory system. When the auditory brainstem response was restored in combination with increased Arc levels, tinnitus was not observed (Singer et al., 2013). These findings provide potential new insights into the molecular basis of tinnitus. If inner hair cell damage is responsible for tinnitus, testing the efferent system with outer hair cell mediated MOC effects, will not test for any role of the efferent system. The inner hair cells are targeted by the lateral olivocochlear (LOC) system. Unfortunately, the LOC system is poorly understood. As with the MOC, the LOC also seems to have a protective effect on the cochlea, but with inner hair cell protection in acoustic trauma (Darrow et al., 2007). This means that to have a thorough understanding of the efferent auditory system in tinnitus, the LOC system targeting the inner hair cells must not be overlooked. But current knowledge of the LOC is too limited to suggest experiments to explore its role in tinnitus directly.

2.5 Conclusion

In conclusion we do not know whether the efferent system is responsible for the origin of tinnitus. But differences in the reactions of the efferent system to cochlear damage could explain why not all people with hearing loss experience tinnitus. Possibly the efferent auditory system is the factor that determines whether a patient's increased spontaneous neural activity after cochlear damage results in tinnitus. It will be worthwhile to investigate the efferent auditory system and its relations to tinnitus. With this paper, we hope to inspire such work.

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Chapter 3

Asymmetry in primary auditory cortex activity in tinnitus patients and controls

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3.1 Introduction

Tinnitus is a phantom sound percept in the absence of an external sound-generating device. Approximately 10-15% of the general population suffers from tinnitus, and 4-5% is severely affected by it (Axelsson and Ringdahl, 1989; Heller, 2003). The perception of tinnitus causes problems with concentration, falling asleep, anxiety and feelings of depression. Hence, tinnitus can have severe negative implications on the perceived quality of life.

There are at least two forms of tinnitus, classified by their characteristics and etiology (Lockwood et al., 2002; Heller, 2003; Møller, 2006). The first form is objective tinnitus, which can be heard by an external observer. It is a very rare form of tinnitus that may be caused by e.g. a vascular or muscular condition (Perry and Gantz, 2000). The second form of tinnitus is subjective tinnitus. In contrast to objective tinnitus, subjective tinnitus can only be perceived by the patient. It cannot be heard by an external observer and no acoustic sound source can be identified. Consequently, subjective tinnitus is a phantom percept. In the current paper, we focus solely on subjective tinnitus, which will be referred to simply as 'tinnitus'.

Subjective tinnitus is believed to be the result of plastic changes and reorganization processes in the auditory pathway and brain structures, most likely caused by the deprivation of input (Møller, 2006). This deprivation of input may result from peripheral hearing loss. In animals studies it has been shown that peripheral hearing loss leads to abnormal spontaneous activity in auditory brain areas. For example, noise-induced hearing loss leads to an increase of spontaneous neural activity in the primary auditory cortex of cats (Norena and Eggermont, 2003), and in the dorsal cochlear nucleus of hamsters (Kaltenbach et al., 2004). It is conceivable that an abnormal increase of spontaneous activity in the auditory system is then perceived as tinnitus.

Several attempts have been made to record this change of activity in the central nervous system that is associated with tinnitus in humans. In their extensive review Lanting et al. (2009) have described the use of different imaging techniques for the registration of this activity with the relevant advantages and disadvantages of the different techniques that are currently available. Traditionally, positron emission tomography (PET) has been used to measure physiological baseline metabolic activity, for example in the diagnosis of oncologic pathology. In the case of tinnitus, it may be expected that if

tinnitus corresponds to enhanced neural activity, this activity would correspond to an enhanced metabolic rate.

Until now 8 studies with [^{18}F]-fluoro-deoxyglucose positron emission tomography (FDG-PET)-scanning in human tinnitus patients have been performed (Arnold et al., 1996; Wang et al., 2001; Eichhammer et al., 2003; Langguth et al., 2003; Langguth et al., 2006a; Smith et al., 2007; Mennemeier et al., 2011; Schecklmann et al., 2013). Arnold et al. (1996) were the first to measure metabolic brain activity in 11 tinnitus patients compared to a control group. They found a significant higher metabolic activity in the primary auditory cortex (PAC), mostly on the left side, versus the other side. The majority of the other studies also showed a higher activity in the left auditory cortex, although in some patients the highest level of metabolism was present on the right side (Arnold et al., 1996; Langguth et al., 2006a; Smith et al., 2007). Recently a large study with 91 tinnitus patients also showed an increase of activity in the left auditory cortex versus the right auditory cortex, but this was not compared with a control group (Schecklmann et al., 2013). Considering that the higher metabolic rate is an indication for increased brain activity, the perception of tinnitus could thus be localized in the left PAC. Unfortunately, all studies mentioned above, except Arnold et al. (1996) and Wang et al. (2001) did not use a control group to compare the found lateralization.

The notion of hyperactivity in the left PAC of tinnitus patients has motivated targeted treatment protocols of repetitive transcranial magnetic stimulation (rTMS) (Plewnia et al., 2003). This experimental treatment modality offers a noninvasive method for altering excitability of the brain (Kleinjung et al., 2005). Langguth et al. (2003) presented a patient with a 4-week reduction in tinnitus sensation after rTMS over the left PAC. Other studies with left-sided rTMS also demonstrated a significant but transient reduction in tinnitus sensation (Eichhammer et al., 2003; Kleinjung et al., 2005; Langguth et al., 2006a; Rossi et al., 2007; Smith et al., 2007; Khedr et al., 2008; Mennemeier et al., 2011). Thus although the previously reported asymmetry in the auditory cortex was not always compared to control subjects, several experimental treatments were aimed at reducing this hyperactivity.

The purpose of the current study was to verify whether left-sided resting-state hyperactivity as recorded by FDG-PET is specifically related to the presence of tinnitus. Thus, we set out to study resting-state metabolic activity by FDG-PET in subjects with bilateral tinnitus, and to compare their results to those of control subjects without

tinnitus. In our analysis, we specifically focused on asymmetries between the left and right auditory cortices in order to allow for a straightforward comparison to earlier studies.

3.2 Experimental procedures

3.2.1 Subjects

Patients were recruited from our tertiary referral outpatient clinic. We included 20 right-handed chronic tinnitus patients (50% male, mean age 51.0 years, standard deviation (SD) 10.0). The mean duration of their tinnitus was 10.5 years (range 1-20 years). Tinnitus was bilateral and constantly present in all cases. Five patients reported an etiology of their tinnitus (loud noise in three, an ear infection in two cases). The tinnitus pitch was assessed by matching it to that of an external tone or 1/3-octave noise band. The median tinnitus frequency was 4000 Hz, ranging from 500 to 11,200 Hz. The tinnitus severity was assessed by the tinnitus handicap inventory (THI; Newman et al., 1996). Mean THI of our subjects was 37.9 (SD 18.5, range 12-86). Patients with any major medical, neurological or psychiatric diagnoses, specific epilepsy, severe head injury or previous cranial neurosurgery were excluded. Tinnitus patients who used drugs or medications that reduced cortical excitation such as anticonvulsants, benzodiazepines or other sedatives (e.g. antihistamines) were also excluded.
For the control group we included 19 healthy subjects, all right handed (47% male, mean age 50.8 years, SD 9.5). Exclusion criteria were equal to those in the tinnitus patients. All selected subjects were right-handed; this was confirmed with the Edinburgh handedness inventory (Oldfield, 1971).

All subjects were examined by an audiologist with a pure-tone audiometry. The pure-tone average (PTA) for the hearing loss was defined as the average hearing threshold at 1, 2 and 4 kHz. The study was approved by the local Medical Ethics committee. We obtained written informed consent from all participants, in accordance with the Declaration of Helsinki (2004). The study was approved by the Medical Ethics Committee of the University Medical Center Groningen.

3.2.2 PET scanning protocol

All the scans were performed dynamically on a Siemens ECAT HR+ PET scanner. After arrival of the tinnitus patient at the scanning room, he or she had to rate the loudness and burden of their tinnitus at that moment on a scale from 0 to 100 (with 0 being

very weak and 100 being very strong). To reduce auditory input, subjects used bilateral earplugs and earmuffs during the experiment. Subjects were placed in the scanner and a lead shield was placed on the subject's chest to reduce radiation artifacts originating from the chest or body of the subjects.
After these preparations ~200 MBq of FDG was injected. The subjects were asked to lie quiet in the scanner for 30 minutes in a quiet and dark surrounding. After this 30-minute uptake time the scanning protocol started, in which the subjects remained lying quietly. The scanning protocol was divided in five blocks of 4 minutes without interruption.

3.2.3 Data analysis

PET images were analyzed using Matlab 7.5 (R2007b) (The Mathworks Inc., Natick, MA, USA) and SPM5 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/). The images were corrected for motion using realignment of all five sets of images to the first volume of each subject. The corrected images were spatially normalized to the SPM PET template. After normalization, the images were smoothed with an 8-mm full width at half-maximum (FWHM) isotropic Gaussian kernel.

3.2.3.1 Voxel wise analysis

For the voxel wise analysis we used the mean images of each subject, obtained in the previous step. Group means were compared using a two-sample *t*-test. We used a relative threshold mask of 0.8 and an implicit mask (excluding voxels with value zero in one of the subjects). Global normalization was performed by a proportional scaling, using a grand mean value of 50 ml/dl/min.

3.2.3.2 Region of interest analysis

We used a Region-of-interest (ROI) analysis for comparison of the activity in selected areas of the brain. ROI analyses were performed on six auditory Brodmann Areas (BAs): BA 41, BA 42, and BA 22, as defined in both hemispheres separately, according to the Wake Forest University (WFU)-pickatlas (Maldjian et al., 2003). In addition, two ROIs were included comprising the left and the right inferior colliculus (IC), respectively. These ROIs were defined by two spheres with radius 5 mm (Montreal Neurological Institute (MNI)-coordinates ±6, -33, -11). For each ROI, the average signal value was calculated for each subject.

3.3 Results

Patients and controls were comparable in age (p=0.973). At the start of the scan session, the mean loudness rating (range 0-100) was 56.7 (SD 21.3) and the burden rating (0-100) was 50.4 (SD 24.6). All patients indicated that they were experiencing their tinnitus during the scanning protocol.

Figure 1 shows the mean hearing loss in both groups. Patients had significantly more hearing loss in comparison with the control group. The mean PTA on the right side for patients was 30.0 dB hearing level (HL), SD 17.6, and for controls 14.3 dB HL, SD 5.3, p=0.001. On the left side the mean PTA for patients was 31.3 dB HL, SD 15.8, and for controls 14.9 dB HL, SD 6.2, (p<0.001).

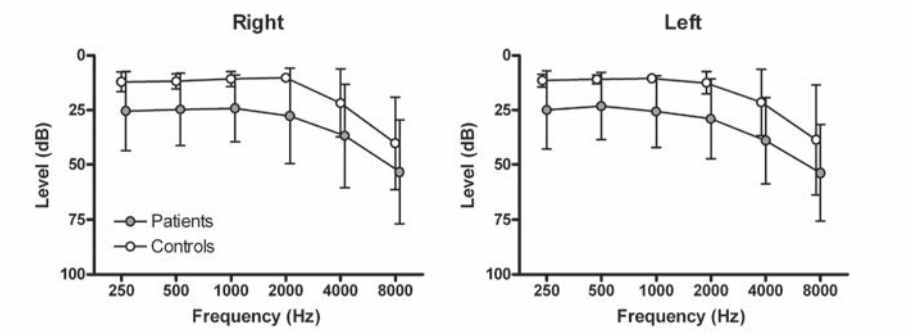

Fig. 1. Mean hearing level (± SD) for the tinnitus patients and the control group.

3.3.1 Voxel analysis

For the voxel analysis we used proportional scaling, $p < 0.001$, uncorrected for multiple comparison and with a minimum cluster size of 50 voxels. With this analysis two clusters of significant difference were detected (see Table 1). The activity of these voxel clusters was lower in tinnitus patients than in control subjects. These areas of differences in PET signal are shown in figure 2, superimposed on a standard magnetic resonance imaging (MRI) image. The largest cluster is shown in panel a, and is part of the left middle frontal gyrus (MFG). The second cluster is shown in panel b, and is located in the left superior temporal gyrus, anterior division (STGa). The analysis was repeated with (1) THI or (2) mean PTA for both ears as a covariate, respectively. Then, all cluster sizes reduced below the 50-voxel threshold, except the cluster in the left MFG with the THI; this cluster reduced in size but remained just above the 50-voxel threshold. No new clusters appeared. When the results were corrected using a Family-Wise Error rate (FWE)

or False Discovery Rate (FDR), no areas of significant difference remained. None of the analyses showed an area of hyperactivity in tinnitus patients.

Table 1. Regions where PET activity was lower in patients than in controls (p<0.001, uncorrected; minimum clustersize 50 voxels). The MNI coordinates (x,y,z) refer to the peak voxel in the cluster, the Z-score is for this voxel. Differences were not significant when corrected for multiple comparisons. Abbreviations: MFG=middle frontal gyrus, STGa=superior temporal gyrus, anterior division.

Region	Hemi-sphere	Peak voxel				Cluster	
		x	y	z	Z-score	Size	p_{unc}
MFG	**Left**	–42	6	32	4.17	127	0.049
STGa	**Left**	–56	0	–6	3.76	93	0.086

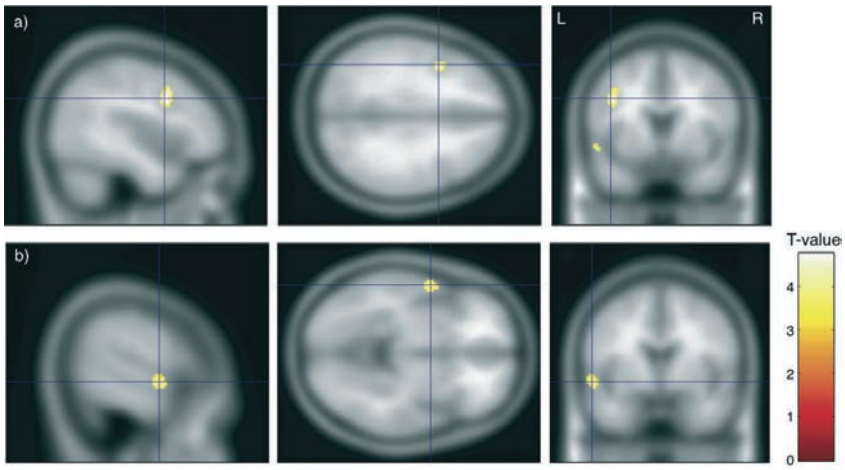

Fig. 2. Two clusters of voxels that showed significant lower PET activity in patients than in control subjects. Panel a shows the largest significant cluster (MNI coordinates -42, 6, 32). This is part of the left middle frontal gyrus (MFG); panel b shows the second significant cluster (MNI coordinates -56, 0, -6) and is part of the left superior temporal gyrus, anterior division (STGa).

3.3.2 Region-of-interest analysis

Figure 3 shows the relative activity of eight different ROIs: bilateral auditory BA 41, 42 and 22, and bilateral IC. There was no evidence in any ROI for hypo- or hyperactivity in tinnitus patients as compared to controls (Student *t*-test, $p > 0.05$). The activity was lateralized to the left in BA 41, and to the right in BA 42 and 22. The IC showed no clear lateralization. There was no significant correlation between the THI and activity in the ROIs for the tinnitus patients. Neither was there a significant correlation between the mean PTA for both ears and the ROI activity, when tested for the tinnitus patients as well as all subjects combined ($p>0.05$).

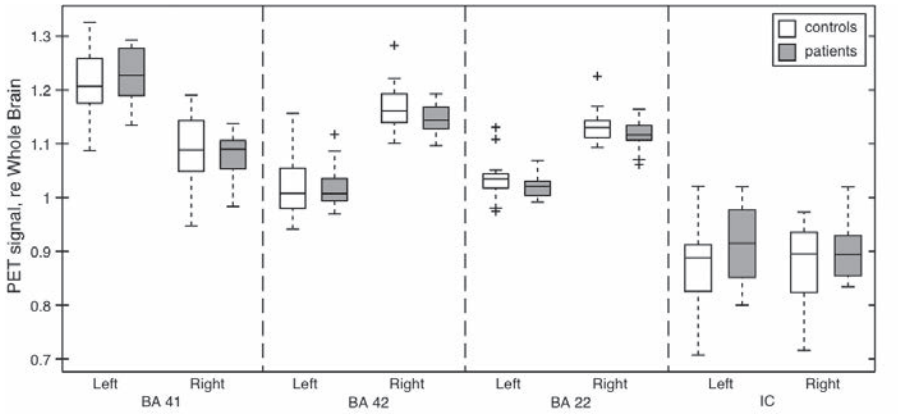

Fig. 3. Box and whisker plots of PET activity measured with ROI analysis in bilateral auditory Brodmann areas (BA) 41, 42 and 22 and the Inferior Colliculus (IC), relative to the mean PET activity in the whole brain for patients and controls. The boxes represent the lower and upper quartile, the bold line the median value. The whiskers represent the extreme data value (within 1.5 times the interquartile range, otherwise denoted by a "+" as an outlier. None of the eight ROIs showed a significant difference between patients and controls.

Figure 4 focuses on the asymmetry of BA 41, 42 and 22. Panels a, c, and e show scatter plots of the mean raw PET signal of the left versus the right hemisphere, for each subject. The activity was lateralized to the left in BA 41, and to the right in BA 42 and 22 in each individual subjects. For comparison with previous studies, we also computed an asymmetry index for each subject and BA: from the mean ROI signal of both hemispheres the asymmetry index was defined as: (left – right)/(left + right). This index is sometimes referred to as laterality index (Seghier, 2008). The right column of Figure 4 (panels b, d, and f) shows this index for the three auditory BAs.

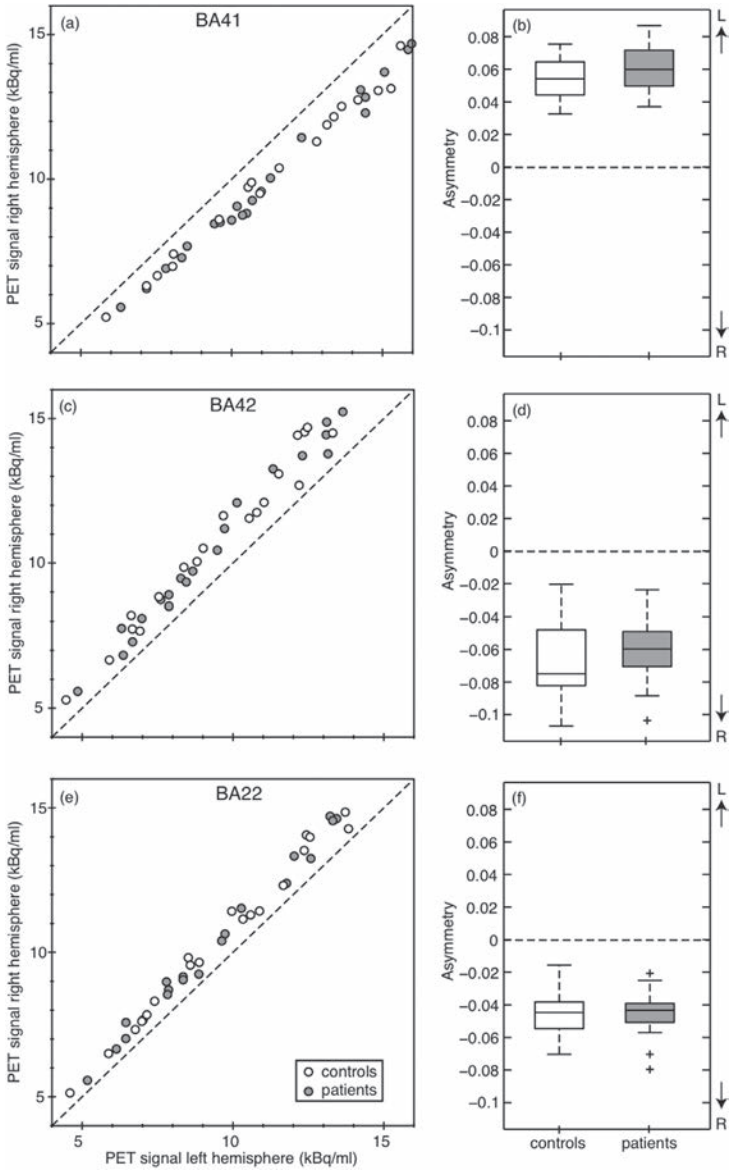

Fig. 4. (a): Mean raw PET signal for left versus right BA 41 (primary auditory cortex). Every point represents one subject. (b): Box and whisker plot for the asymmetry index of the data points from panel (a). Positive asymmetry indicates a higher activity in the left hemisphere; Panels (c) and (d): Same for BA 42 (secondary auditory cortex); Panels (e) and (f): Same for BA 22 (auditory association cortex).

3.4 Discussion

FDG-PET scanning of the resting-state metabolism of the auditory cortex showed no evidence for hyperactivity in tinnitus patients. We consistently found an asymmetry in the resting-state metabolism of the auditory cortex. Our data show that this asymmetry in the auditory cortex is also present in control subjects, and is not exclusive for tinnitus patients. For the primary auditory cortex the activity was higher on the left side, in line with previous reports in tinnitus patients. In secondary and association auditory areas we also found asymmetrical activity, however here the activity was higher on the right side. The asymmetry was not significantly different between tinnitus patients and controls. Thus, we could not confirm that tinnitus is associated with left-sided cortical hyperactivity. However, we found two areas with reduced activity in tinnitus patients, compared with control subjects: part of the anterior division of the left superior temporal gyrus, and an area in the left middle frontal gyrus.

The higher metabolism in the left PAC is in line with previous reports (Arnold et al., 1996; Langguth et al., 2006a). Both Arnold et al. and Langguth et al. reported an asymmetry index to compare activity in left and right PAC. Figure 5 shows boxplots of the PAC asymmetry index of all subjects from these two papers and the current report. Schecklmann et al. (2013)and Smith et al. (2007) did not report the amount of the asymmetry, so we could not include their data in fig. 5. In the included papers, all subjects except three show a positive asymmetry, i.e., higher activity in the left PAC. Important to note is that Arnold et al. (1996) used an absolute value for the asymmetry index, resulting in a positive number. The five FDG-PET studies (Arnold et al., 1996; Langguth et al., 2006a; Smith et al., 2007; Schecklmann et al., 2013) and ours) agree in that they all report a left hemisphere lateralization of resting-state activity of the PAC in tinnitus patients.

Only Arnold et al. (1996) and the present study included control subjects, but the outcomes of the controls are different. Arnold et al. (1996) showed almost symmetric activation in the PAC in control subjects. In contrast, our control subjects showed left-lateralized resting-state activation in PAC, similar to that in tinnitus patients (Fig. 5). A potential explanation for this difference may be related to the way subjects were isolated from ambient sound in the scanner room. During our scanning protocol all subjects had both earplugs and earmuffs. In the study of Arnold et al. (1996), only six of the 14 control subjects (and all patients) used earplugs during the scan. The other eight

subjects did not wear sound isolating earplugs. It is possible that accidental sounds during the scanning protocol gave a rise in the PAC metabolism in both hemispheres of these subjects, resulting in symmetrical activation. Also, our ROI definition of the PAC was different from the one Arnold et al. (1996) used. They defined the PAC by four circular slices covering the temporal transversal gyrus, whereas we used the WFU-pickatlas (Maldjian et al., 2003). Although these ROI definitions are different, it cannot explain why only the controls (and not the patients) from Arnold et al. (1996) differ from our subjects.

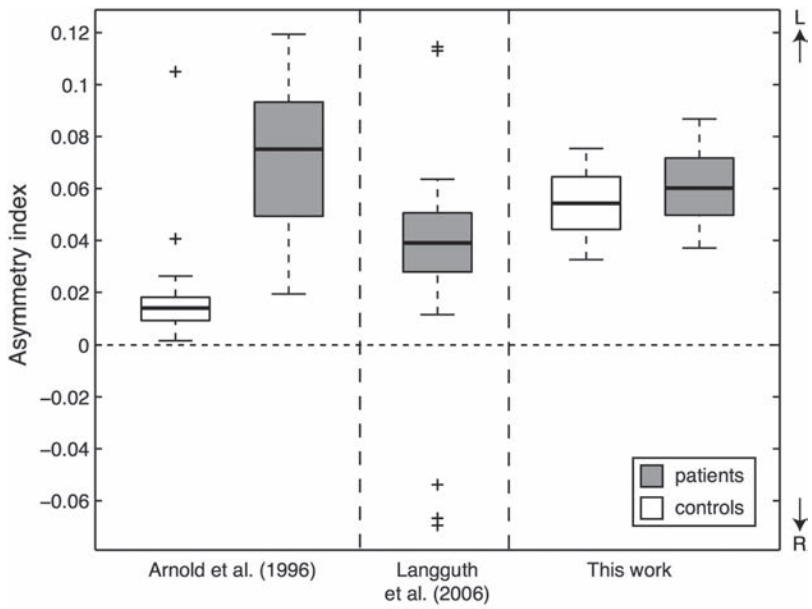

Fig. 5. Comparison of the asymmetry index of the primary auditory cortex in different FDG-PET scan studies. The data of Arnold et al. (1996, patients N=11, controls N=14) and Langguth et al. (2006a, patients N=20) are presented in way that matches our calculation of the asymmetry index. Again, the boxes represent the lower and upper quartile, the bold line the median value. The whiskers represent the extreme data value (within 1.5 times the interquartile range, otherwise denoted by a "+" as an outlier.

In our data the secondary and association auditory areas showed an obvious lateralization to the right. Remarkably, the asymmetry was opposite to the lateralization we found in the PAC. We also tested for asymmetry in the IC, because of its previously demonstrated role in tinnitus (Melcher et al., 2000; Lanting et al., 2008; Lanting et al., 2009; Melcher et al., 2009). We found no asymmetry and no significant difference in the ICs activity between patients and controls, which is possibly due to the large spread of activation estimates in these small brainstem structures.

Asymmetry in the auditory cortex has been known for a long time, both anatomical and functional (for example see Galaburda et al., 1978, for review see Tervaniemi and Hugdahl, 2003). With functional differences the left temporal lobe is more involved in speech processing, and the right temporal lobe (although to a lesser extent) more in music processing. More in detail, Bernal et al. (2004) have studied the asymmetry in activation of the primary and secondary auditory cortex with an auditory stimulus consisting of repetitive and non-repetitive piano tones, and functional MRI. They found a dominant activation to the left PAC (BA 41), and to the right side for association auditory cortex (BA 22). BA 42 (secondary auditory cortex) was not obviously lateralized in their study (Bernal et al., 2004). The present study showed that in addition to these functional asymmetries, also resting-state metabolic activity is asymmetric in auditory brain areas. As is the case for the functional asymmetries, also the resting-state asymmetries differ across BAs.

The supposed association between tinnitus and left-sided hyperactivity, has motivated experimental rTMS treatment targeted at the left auditory cortex (e.g. Eichhammer et al., 2003; Kleinjung et al., 2005; Langguth et al., 2006a; Langguth et al., 2006b; Rossi et al., 2007; Khedr et al., 2008; Anders et al., 2010; Mennemeier et al., 2011). To influence this presumed hyperactivity, precise localization of the target for rTMS treatment is believed to be necessary. Due to technical limitations, the figure-8 shaped TMS coil generates only a small area of effective magnetic field. Recently it was demonstrated that neuronavigated localization with FDG-PET was not superior to rTMS with coil positioning by electroencephalography coordinates (Langguth et al., 2010; Langguth et al., 2012). Our results now show that this left-sided hyperactivity in the primary auditory cortex seems to be a normal physiological phenomenon present in normal controls as well as tinnitus patients. Hence, then the treatment of tinnitus by rTMS of the left PAC seems to have no physiological basis in terms of asymmetric hyperactivity related to tinnitus. Perhaps the reported –mostly small– treatment effects of TMS on the left primary auditory cortex are not based on reducing the hyperactivity. This may provide an explanation for the finding of Mennemeier et al. (2011), who have shown that the effect of rTMS was not correlated with changes in PET activity at the treatment site.

Contrary to our expectation, we did not find areas hyperactivity related with tinnitus. Nevertheless, several studies suggest an association between tinnitus and hyperactivity (Eggermont and Roberts, 2004; Moller, 2007; Roberts et al., 2010; Kaltenbach, 2011). Alternatively, it has been suggested that increased synchrony rather than increased rate of spontaneous activity is related to tinnitus (Norena and Eggermont, 2003; Norena and

Farley, 2013). Increased synchrony relates to an increase of synchronous activity across neurons in the auditory cortex. It is likely that increased synchrony does not involve an increase in metabolic rate, as the overall rate may be unchanged. Then, it would not be detected by FDG-PET. Thus, our results could be interpreted as being consistent with a relation between tinnitus and increased synchrony of spontaneous activity.

With voxel-wise analysis we found two areas of possible reduced activity. Although these areas were only detected when the analysis was uncorrected for multiple comparisons, they may well be linked to previous findings. The first area is located in the anterior division of the STG. The STG is suggested to be involved in pitch patterns and melody or song, with a small preference for the right hemisphere (Zatorre et al., 1998; Patterson et al., 2002; Brown et al., 2004; Puschmann et al., 2010; Tierney et al., 2013). It is a higher-level auditory area. Its role in tinnitus is not yet clear, but has been reported previously in a tinnitus suppression study (Mirz et al., 2000). Langers et al. (2012) reported on an area close to it. They found enhanced sound-evoked activity in this area for tinnitus patients compared to control subjects. Possibly, the lower activity in a non-stimulated condition (this work) corresponds to the higher response to sound when stimulated (work of Langers et al., 2012), before reaching the maximum activation of that region. Husain et al. (2011) reported on this area in an auditory study as well. They measured gray matter decreases in the superior temporal gyrus in both hemispheres, but this was more related to hearing loss than tinnitus. In close proximity to the superior temporal gyrus is the temporal pole. The temporal pole is considered a paralimbic region, with its connections to the hippocampus and amygdala for visceral emotional responses. Perhaps our location of diminished FDG-uptake in the anterior part of the temporal lobe is a reflection of the emotional disturbances experienced by chronic tinnitus patients, maybe as a cause or as a consequence of tinnitus (Rizzardo et al., 1998). Although the brain areas discussed in these studies do not exactly overlap, they suggest a role of the lateral-anterior portion of the left superior temporal lobe in the perception or the experienced disturbance in tinnitus.

The second area that was hypoactive in tinnitus patients was located in the left MFG. Recently, Golm et al. (2013) also identified this area with functional MRI in tinnitus patients. They found higher activation in the left MFG in highly distressed tinnitus patients when compared to healthy controls. Furthermore, they were able to correlate the measured brain activity with tinnitus-related distress. In our patient group the mean THI was 37.9 (SD 18.5), usually interpreted as a moderate handicap (Newman et al.,

1996). Potentially, the hypoactivity in the MFG is a reflection of the patients' tinnitus distress. As part of the frontal cortex, the lower activity in the left MFG might also be a reflection of the suggested "noise-cancellation" mechanism, in which the limbic regions of the brain fail to block the increased activity after noise trauma to reach the auditory cortex (Rauschecker et al., 2010). One could speculate that this area of lower activity might be a new target for localized neuromodulatory treatment modalities, as recently suggested by Golm et al., (2013).

A limitation of our study is the difference in hearing level between the patients and controls. The tinnitus subjects had on average a mild sensorineural hearing loss (~30 dB), while the control subjects had near normal hearing (~15 dB). When using hearing loss as a covariate in the voxel analysis, both clusters were no longer significantly different between patients and controls. Although we plugged the ears of all subjects, and we did not use sound stimuli, it is conceivable that small hearing loss in the tinnitus subjects influences the metabolic activity in the auditory cortex. With this study patients and controls were not matched for their hearing loss, which resulted in a difference in mean hearing thresholds between both groups and a relatively large standard deviation in the patient group. Effects of hearing loss on the central auditory system are previously reported. For example, several voxel based morphometry (VBM) studies reported gray matter changes in subjects with hearing loss (e.g. Husain et al., 2011; Boyen et al., 2013). However, preservation of gray matter volume was also reported (e.g. Li et al., 2012). Therefore, we cannot exclude the possibility that the small differences in metabolic activity in the left MFG and the STGa are caused by hearing loss rather than tinnitus.

3.5 Conclusion

In conclusion, we have shown that FDG-PET activity in the left PAC was higher than that in the right PAC, in all subjects. This asymmetry was equally present in both tinnitus patients and control subjects and can therefore not be the result of tinnitus. Furthermore, the metabolism of the secondary and association auditory cortices was also asymmetrical, but opposite to the primary region: here the activity was higher on the right side in tinnitus patients and controls. There was no evidence of hyperactivity in the auditory cortex of tinnitus patients. These findings can have consequences for the rationale of treatment protocols for tinnitus based on the concept of the lateralized hyperactivity.

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Chapter 4

Contralateral suppression of otoacoustic emissions in tinnitus patients

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4.1 Introduction

Otoacoustic emissions are weak sounds generated in the inner ear (Kemp, 1978). The influence of contralateral acoustic stimulation (CAS) on the amplitude of otoacoustic emissions (OAEs) was described a decade after their discovery (Mott et al., 1989). In healthy subjects, contralateral acoustic stimulation suppresses the amplitude of OAEs. Spontaneous emissions as well as transient-evoked (TEOAEs) and distortion-product emissions (DPOAEs) are affected by CAS (Mott et al., 1989; Collet et al., 1990). The amount of suppression depends on the intensity that is used for both the OAE stimulus and the CAS (Berlin et al., 1993; Hood et al., 1996). Suppression of OAEs is mediated by the medial olivocochlear efferent system (MOC). Thus, this phenomenon of suppression provides an unique non-invasive tool to study the function of this efferent auditory system (Guinan, 2006).

Our interest in the contralateral suppressive effect was raised by the fact that the efferent system is thought to play a role in tinnitus (Jastreboff, 1990; Bauer, 2004). Tinnitus is a sound percept in absence of an external source. Several hypotheses about the cause of tinnitus have been stated. In general, most concern the changes in the function of the central auditory nervous system, with an altered balance between inhibition and excitation, and the possible reorganization of tonotopic maps (Moller, 2007). Specifically, tinnitus may be related to enhanced activity in the central auditory system. The efferent system is thought to have a role in down-regulation of activity from the auditory system. However, the role of the efferent system in the etiology of tinnitus remains unclear (Jastreboff, 1990; Bauer, 2004). If tinnitus is indeed related to a reduced efficiency of inhibition, the presence of tinnitus may correspond to a reduced effectiveness of the efferent system.

The medial olivocochlear efferent system (MOC) is part of the efferent auditory system. It is responsible for the reduction in signal amplitude of click-evoked OAEs (CEOAEs) during acoustic stimulation. Activation of the MOC occurs when sound is presented to the contralateral ear. The efferent nerve fibers originating in the medial olive, terminate on the outer hair cells and modify their action (Warr and Guinan, 1979). More specifically, activity of these fibers causes hyperpolarisation of the outer hair cell, which alters the action of the outer hair cells on the basilar membrane, and hence changes the so-called cochlear amplifier. As a consequence the OAEs are altered as well (Guinan, 2006). Possibly, reduced central inhibition correlates with reduced inhibition in the MOC. Therefore, tinnitus patients might display reduced suppression of OAEs with CAS.

In tinnitus patients the efferent auditory system has been studied previously with contralateral suppression of CEOAEs. Multiple studies have been published, but results are inconclusive or contradictory (Chery-Croze et al., 1993; Chery-Croze et al., 1994a; Chery-Croze et al., 1994b; Graham and Hazell, 1994; Attias et al., 1996; Lind, 1996; Ceranic et al., 1998; Favero et al., 2006; Riga et al., 2007; Granjeiro et al., 2008). In the first study done by Chéry-Croze et al., (1993) the contralateral ear of the patient was used as a control measurement. Following studies also used the contralateral ear of the tinnitus patient for control measurements (Chery-Croze et al., 1994a; Chery-Croze et al., 1994b; Lind, 1996; Riga et al., 2007). These studies reported less suppression or found no significant difference in suppression between the "tinnitus ear" and the "normal" contralateral ear. However, the contralateral ear may not be suitable as a control measure: although patients might perceive their tinnitus as unilateral, the underlying pathology (hyperactivity in the brain) may not be lateralized (Lanting et al., 2008). Also, hearing in the tinnitus ear is in general worse than in the contralateral ear. Few studies have used a control group to compare suppression (Graham and Hazell, 1994; Attias et al., 1996; Ceranic et al., 1998; Favero et al., 2006; Riga et al., 2007). This suggests that tinnitus patients have less suppression than healthy controls. However, the research designs were not always clear about signal-to-noise criteria in the data selection, or the amount of suppression.

With the technical limitations of the published reports, we felt the need to repeat a study for the effectiveness of the MOC in tinnitus patients. The purpose of this study was to compare the amount of contralateral suppression of tinnitus patients to a control group. We selected these subjects on the presence of otoacoustic emissions as determined by a clear signal-to-noise criterion. We tested the hypothesis that the contralateral suppression of otoacoustic emissions was similar in tinnitus patients compared to a control group.

4.2 Material and Methods

4.2.1 Otoacoustic emissions

CEOAEs were recorded and analyzed using the Otodynamics Ltd ILOv6 (United Kingdom). We started with standard CEOAE measurements, with a peak stimulus level of 80 dB SPL (see Figure 1). For each ear, the responses of at least 300 sets of 4x2 stimuli (i.e. three in phase, one in opposite phase with triple amplitude; in buffers A and B) were

averaged in about 70 seconds, in order to obtain the CEOAE signal. Emissions were recorded using the standard non-linear stimulation method. The artifact rejection level was 50 dB SPL. If this resulted in a whole-wave reproducibility of less than 50%, the ear was excluded for further analysis. Both ears were regarded separately, so suppression was sometimes measured only in 1 ear of an individual, independent of tinnitus side.

Ears with reproducible CEOAEs were subsequently tested for contralateral suppression of the emission. The peak stimulus level was 65 dB SPL and a total of at least 500 sets of 4x2 click-responses were averaged in order to obtain the response, for the suppressed as well as the unsuppressed CEOAE. Both conditions were measured interleaved, in about 230 seconds. Again, emissions were recorded using the standard non-linear stimulation method. Contralateral broadband noise was presented at 70 dB SPL. Again, CEOAEs were recorded in response to stimulus clicks with the artifact rejection set at 50 dB SPL. No additional testing was performed to ensure that middle-ear muscle (MEM) activity would not influence the data, considering that this activity is most pronounced at sound levels of 75 dB and higher, and would be equal in both groups.

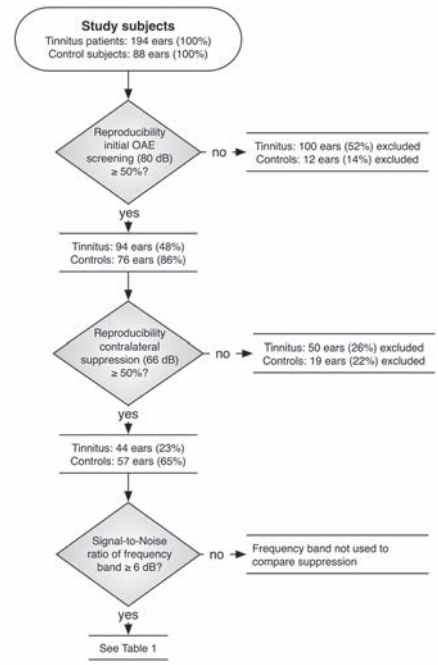

Figure 1. Flow-chart with the study protocol and number and percentage of included ears for both groups.

The ILO device calculated the total broadband CEOAE response and noise level, as well as in five half-octave frequency bands, centered at 1.0, 1.4, 2.0, 2.8 and 4.0 kHz. The suppression was calculated by subtracting the CEOAE signal amplitude with CAS from the signal amplitude without CAS, for each frequency band. Suppression in a particular frequency band was only considered if the CEOAE signal met 2 criteria: (1) the whole-wave reproducibility for the unsuppressed CEOAE was 50% or better, and (2) the signal-to-noise ratio (SNR) in the band was 6 dB or higher, for either the suppressed or unsuppressed OAE. The inclusion protocol together with the number of ears included in each step is shown in Figure 1.

4.2.2 Subjects

Ninety-seven consecutive tinnitus patients who visited our specialized out-patient tinnitus clinic were investigated with the initial OAE screening (65% male, mean age 54 years, SD 12 years). Median tinnitus duration was 4.0 years, and ranged from 1 to 55 years. The tinnitus was left-sided in 33%, right-sided in 32% and non-lateralized in 35% of the tinnitus patients. The causes of the tinnitus reported by the patients were an ear infection in 6 cases, a generalized illness in 4 cases, psychiatric distress in 7 cases, following an accident in 7, of which 1 case of whiplash. Thirteen patients reported a loud sound as the cause, and 3 complained of tinnitus after an episode of idiopathic sudden sensorineural hearing loss. In addition, 19 of the 97 patients also reported the use of medication, known for ototoxicity or tinnitus as a side effect. The cause was unknown in 72 of 97 cases.

All subjects were screened with standard pure tone audiometry, otoscopy, a tinnitus masking test and CEOAEs. One hundred ears (49 right, 51 left) of the initially analyzed patients did not reach the level of 50% whole-wave reproducibility of the CEOAE in response to a 80-dB SPL click stimulus and were excluded. So, contralateral suppression measurement was done in 94 ears (48 right, 46 left). The mean age of the included tinnitus patients was 50 years (SD 12) with 61% males.

The control group consisted of 44 subjects without tinnitus. All 44 subjects were screened with standard pure tone audiometry and CEOAEs. As with the patient group, only ears with a whole-wave reproducibility of 50% or more of their CEOAE signal were included for the contralateral suppression measurement. The included control group contributed 76 ears (36 left, 40 right). So 12 ears (4 right, 8 left) were excluded from the control group. The control group consisted of 43% male, with a mean age 46 years (SD 10).

For analysis of the effect of contralateral stimulation, only the subjects with at least 50% reproducibility in response to a 65 dB SPL stimulus were used to calculate the suppression. This resulted in 44 ears (23 right, 21 left) for the patient group and 57 ears (31 right, 26 left) for the control subjects. In this group the mean age of the tinnitus patients was 48 years (SD 10) with 63% male and for the control group 44 years (SD 10) with 35% male. The tinnitus was lateralized to the right in 7 ears, to the left in 11 ears, and not lateralized in 12 ears. The median tinnitus duration in the patient group was 3.0 years, and ranged 1 to 30 years. The pure-tone average (PTA) was defined as the average hearing threshold at 1, 2 and 4 kHz.

4.2.3 Statistical analysis

Preliminary analysis showed that the data were not normally distributed. Therefore, we used non-parametric testing for two independent samples (Wilcoxon-Mann-Whitney rank-sum test), or related samples when necessary, in SPSS software (SPSS 14.0 Inc. Chicago). For the testing of correlation we used the Pearson R correlation coefficient. We defined statistical significance as a probability (p) value of <0.05. All procedures were in accordance with the declaration of Helsinki.

4.3 Results

Mean age was not significantly different for the included patient and control group ($p = 0.172$ according to Wilcoxon-Mann-Whitney test). The percentage of males was significantly higher in the patient group ($p = 0.026$). The amount of suppression of all included frequency bands was tested for correlation with age. There was no significant correlation between age and suppression in patients (Pearson R=-0.21, p=0.16) and controls (Pearson R=0.12, p=0.33). Also, there was no correlation between age and suppression for the separate frequency bands (e.g. suppression in the 1.0-kHz band correlated with age, suppression in the 1.4 kHz-band correlated with age, etc.). The noise levels as well were not significantly different between both groups, in the condition with and without CAS. The SNR was only significantly different for the 4.0 kHz frequency band in the right ear ($p = 0.04$).

Hearing loss was significantly different between patient and control group. Figure 2 shows the average pure-tone audiograms for these subjects, which were included with 50% or more whole-wave reproducibility of the CEOAEs with the 65-dB click stimulus. The

PTA for the patients with tinnitus was 17.4 dB (SD 12.8) for the right ear and 19.4 dB (SD 12.3) for the left ear. For control subjects the PTA was 11.5 dB (SD 2.6) for the right ear and 12.1 dB (SD 3.1) for the left ear. The control group had significantly less hearing loss than the included tinnitus patients ($p < 0.012$ for the right ear and $p < 0.005$ for the left ear).

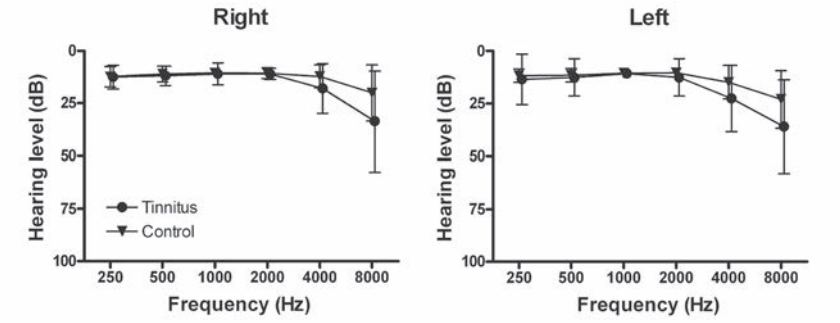

Figure 2. Standard pure tone audiogram (mean and standard deviation) for tinnitus patients with CEOAEs and for control subjects. Only the subjects that met the criteria at the 65 dB stimulus level are included in this figure.

Figure 3 shows the CEOAE amplitude for the 65 dB stimulus without CAS for these two groups. The amplitude in each of the 5 frequency bands was compared between the two groups. There was no significant difference in the amplitude of the emission signal between the tinnitus patients and the control subjects, except for the 4.0 kHz frequency band on the right side (p-value 0.034), which is probably due to the poor SNRs at 4 kHz. There was no significant difference in the amplitude of the CEOAE between right and left ears, for both patients and control subjects.

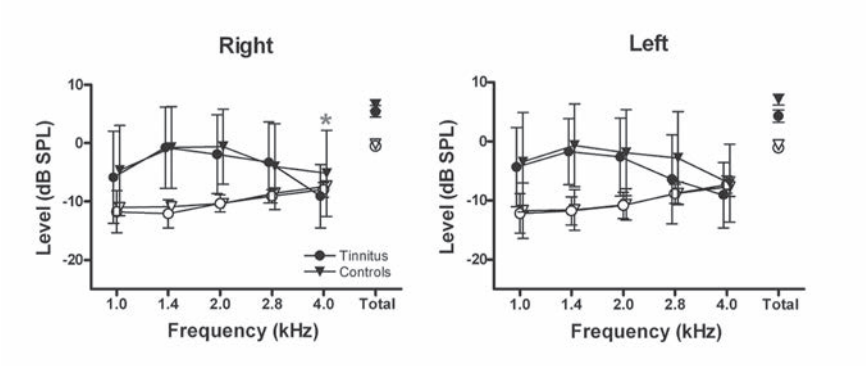

Figure 3. The mean amplitude (and standard deviation) of the CEOAE signal and noise, per frequency band; total indicates the broad band OAE level. CEOAE data are measured without contralateral acoustical stimulation with a 65 dB stimulus level, for patients (44 ears) and control subjects (57 ears). Significant difference is marked with an asterisk. Closed symbols: signal; open symbols: noise.

Figure 4 shows the CEOAE amplitude with and without CAS, for the patient and control group. For each included ear, the (suppressed and unsuppressed) data for a frequency band were included when either the suppressed or unsuppressed OAE had a SNR of 6 dB or more. So the mean amplitude per frequency differs from the amplitude in Figure 3, for which all bands were included. As a result of this selection, the number of included signals differed per frequency band (see Table 1). Suppression of the signal was statistically significant for both tinnitus patients and controls, for all frequencies except the 4.0-kHz frequency band on the left side for controls, and the 2.8 kHz frequency band for the patients on the left side (for 4.0 kHz only one tinnitus patient was included for both sides, so this could not be tested statistically with a paired sample Wilcoxon-Mann-Whitney test).

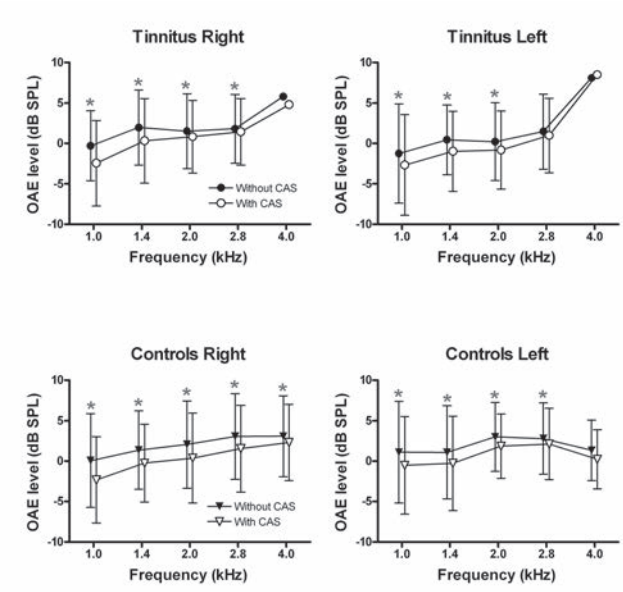

Figure 4. The mean amplitude (and standard deviation) of the CEOAEs with and without CAS, for tinnitus patients and control subjects. Only frequency bands with a SNR of 6 dB or more were included (see Table 1). Suppression of CEOAEs was present for tinnitus patients and control subjects, and was statistical significant for almost all frequencies (marked by asterisk).

Table 1. Number of included ears per frequency band, with a SNR of 6 dB or higher: the mean amplitude of the CEOAE without CAS is indicated (cf. Figure 4).

	OAE amplitude (dB SPL)							
	Controls Right ear		Patients Right ear		Controls Left ear		Patients Left ear	
Frequency (kHz)	Ears	Amplitude	Ears	Amplitude	Ears	Amplitude	Ears	Amplitude
1.0	16	0.1	13	-0.3	17	1.1	13	-1.3
1.4	26	1.4	18	2.0	21	1.1	16	0.5
2.0	22	2.1	15	1.5	15	3.0	15	0.2
2.8	12	3.1	12	1.8	14	2.8	7	1.5
4.0	10	3.1	1	5.8	7	1.3	1	8.1

The amount of suppression is shown in Figure 5. The calculated average suppression for all frequency bands was larger than zero, for both tinnitus patients and control subjects. This was the case, except for the one left ear of a tinnitus patient for the 4.0 kHz frequency band. This ear had negative suppression, i.e. enhancement of the CEOAE. Thus suppression was present in both tinnitus patients and control subjects. There was no significant difference in the amount of suppression between tinnitus patients and control subjects, except for the right ear in the frequency bands centered at 2.0 and 2.8 kHz, with a p-value of 0.03 and 0.008, respectively. The amount of suppression in those frequency bands was less for tinnitus patients as compared to controls.

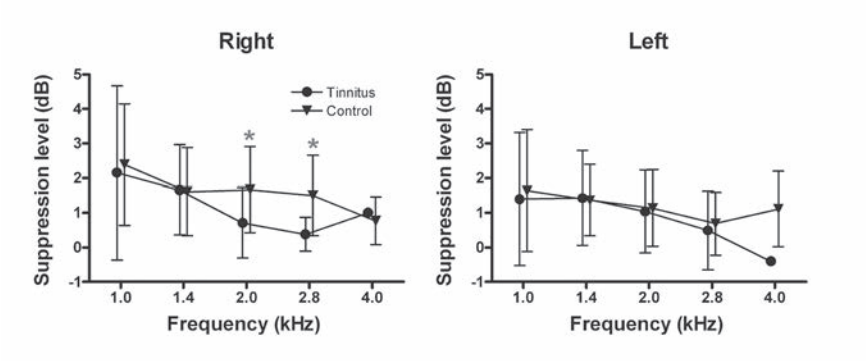

Figure 5. The amount of suppression, calculated as the difference in amplitude between the CEOAE with and without CAS, for tinnitus patients and control subjects. The amount of suppression is comparable for tinnitus patients and control subjects for most frequencies (significant difference marked by asterisk).

4.4 Discussion

We selected tinnitus patients and control subjects on having reproducible CEOAEs at 65 dB stimulus level. Our results showed that the amplitude of the CEOAEs was similar in tinnitus patients and the control group. Also, both tinnitus and control subjects showed clear contralateral suppression of CEOAEs. These results contrast previous reports in two ways.

First, the amplitude of the OAEs with and without CAS did not differ significantly between tinnitus patients and control subjects, as can be seen in Figure 3. This is in contrast with the paper by Ceranic et al. (1998) on tinnitus patients with head trauma. Within a group of whiplash subjects, they reported higher OAE amplitudes in the subjects with tinnitus compared to those without tinnitus. Presumably, the difference between Ceranic's result and our data relates to the etiology of the tinnitus in the study group: we selected patients from a general tinnitus population, whereas Ceranic et al. specifically tested tinnitus patients with head trauma (Ceranic et al., 1998). The difference underlines the possibility that tinnitus may be based on a range of different pathophysiological mechanisms.

Second, we showed in this paper that both the tinnitus patients and the control group clearly showed suppression during CAS. Except for the 2.0 and the 2.8 kHz frequency bands in the right ear, the suppression was equal between both groups. The fact that both our groups showed suppression during CAS is in contrast to previous reports: the four previous studies that used a control group reported less or no suppression in tinnitus patients (Attias et al., 1996; Ceranic et al., 1998; Favero et al., 2006; Riga et al., 2007). We suggest that technical differences between the previous studies and ours may account for this difference.

Attias et al. (1996) found less contralateral suppression in patients with (noise induced) tinnitus, with slight enhancement of the signal only in the patient group at low intensity contralateral stimulation. They measured contralateral suppression as a percentage of change of the SNR. A measurement prior to contralateral stimulation was used as a baseline, and was compared to the measurement during contralateral stimulation. The rationale for this technique was to cancel out potential group differences in the absolute amplitude. However, no statement was made about the levels of noise in the measurement. Consequently, it is unclear to what extend measurement noise

may have affected the results. Riga et al. (2007) reported no significant suppression of DPOAEs in acute tinnitus which was in contrast to the control group, but they did not compare the amount of suppression between both groups. Favéro et al. (2006) calculated the amount of suppression by subtracting the SNR without CAS from the SNR with CAS, but without control of the level of noise. Graham and Hazell (1994) tested the variability of the OAE suppression in a small tinnitus patient group. This was larger in the patient group than in the control group. Also, there was a trend for lesser suppression of transient evoked OAEs in the patient group, but this was not significant (Graham and Hazell, 1994). So our finding of significant suppression of OAEs in tinnitus patients remains remarkable considering the currently available published literature.

The difference between our and other studies may be due to the patient selection criteria we applied; our patients were drawn from a general tinnitus population. For inclusion, the recorded OAE signal had to be 6 dB above the noise floor, for the unsuppressed or the suppressed condition. With this selection of true OAE-signals, we are confident to state that these tinnitus patients do have suppression of CEOAEs, indicating a functioning MOC. Also the amount of suppression of the OAEs with CAS of both groups is in line with previous findings from normal hearing subjects (Berlin et al., 1993; Hood et al., 1996), where the amount of suppression varies between 0.5 dB and 3 dB. This suggests that our signal selection criteria adequately distinguished between emission and noise components in the recorded signals and the amount of suppression is comparable to normal hearing subjects.

The majority of the frequency bands for the amount of suppression were statistically not significantly different between patients and control subjects. But 2 frequency bands of the right ear, centered around 2.0 and 2.8 kHz, were significantly different. So, although tinnitus patients have suppression, it is not for all frequencies the same amount as the control subjects. It is unclear to us why only these 2 frequency bands in the right ear showed a significant difference. Usually, suppression is more pronounced in the lower frequencies, so we would expect that differences in suppression would be more obvious in the 1.0 and 1.4 kHz frequency bands (Veuillet et al., 1992; Morand et al., 2000). The other striking part of these findings is that only the 2 bands in the right ear were different, and not the left ear. We do not have an explanation for this difference with regard to lateralization of the tinnitus or the amount of hearing loss. One other potential explanation might be the limitations of the statistical analysis. Because multiple comparisons were made in the two groups, the statistical significance might have been

caused by a Type I error. Theoretically, with a p-level of 0.05, the chance of getting two false positives out of 10 comparisons equals 0.05^2*0.95^8*10!/(8!*2!)=0.075.

To assess the role of the efferent auditory system in tinnitus, one should ideally investigate the entire efferent pathway, which runs from the auditory cortex to the cochlea (Suga and Ma, 2003). In humans this research is restricted with obvious ethical and legal boundaries. Recently, 3 papers have been published with results from the corticofugal system, studied indirectly (Khalfa et al., 2001; Perrot et al., 2006; de Boer and Thornton, 2007). These papers demonstrated the functional existence of the corticofugal pathways in humans, as well as the influence of the cortex on the MOC. Our results imply that the MOC is functional in tinnitus, although minor differences exist between tinnitus and controls subjects. Note that our results do not allow for any conclusions about the remaining efferent auditory system, which may still play a part in the pathophysiology of tinnitus.

A limitation of studying the inhibitory capacity of the central auditory system with OAEs is that subjects must have functional outer hair cells to be able to produce any OAEs. Obviously, our findings are limited to those patients that do have significant OAEs. The majority of our initially analyzed tinnitus population had significant hearing loss and absent or weak OAEs. Consequently, only 30 patients (with a total of 44 suitable ears) of the original 97 tinnitus patients could be used for the final analysis. This limitation makes the OAE-measurement not suitable for generalization of the results for the entire tinnitus population, but confines the conclusion to the tinnitus population with detectable OAEs. Another limitation of our study is the difference in hearing loss between the subject groups. As can be seen in Figure 2, the tinnitus subjects have significant more hearing loss in the higher frequencies than the control subjects. The only difference we found between the subject groups was a slightly reduced suppression in the right ear of tinnitus patients. Potentially, this slight left-right asymmetry can be accounted for by asymmetries in the tinnitus group. However, there was no difference between the hearing loss in the left and the right ear and the lateralization of tinnitus was balanced within the tinnitus group. Thus, the slight asymmetry in amount of suppression cannot be accounted for by asymmetries in other audiometric parameters. The asymmetry suggests a subtle difference in the function of the MOC between tinnitus and control subjects.

In summary, we conclude that contralateral suppression of otoacoustic emissions for tinnitus patients is present and within normal ranges. Compared to a control group, suppression was equal, except for two out of five frequency bands (centered at 2.0 and 2.8 kHz) in tinnitus patients' right ears, where suppression was less pronounced. Apparently, the MOC is functional in tinnitus patients in suppressing the activity of the outer hair cells and basilar membrane vibration. The minor difference between tinnitus and control subjects suggests subtle differences between both study groups. A role for the medial olivocochlear efferent system in the etiology of tinnitus still remains possible.

Acknowledgments

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Chapter 5

Wavelet-analysis demonstrates no abnormality in contralateral suppression of otoacoustic emissions in tinnitus patients

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5.1 Introduction

Tinnitus is the very frequently heard complaint of the perception of a sound in absence of an external source. Several hypotheses exist on its origin. In general, they concern the altered balance in the central auditory system, with reorganisation of tonotopic maps (Moller, 2007; Roberts et al., 2010). Changes in the balance between excitation and inhibition are likely to play a role. Because the efferent auditory system is thought to participate in down-regulation of activity in the central auditory system, abnormal function of this system could possibly contribute to the origin of tinnitus (Jastreboff, 1990; Bauer, 2004).

The function of the most peripheral portion of the efferent auditory system can be tested in humans, by means of contralateral suppression of click-evoked otoacoustic emissions (OAEs). The amount of OAE amplitude reduction provides a unique non-invasive measure of the function of peripheral part of the efferent auditory pathway in humans. The medial olivocochlear (MOC) efferent system is responsible for reduction of click-evoked OAEs. Efferent nerve fibres, originating in the medial olive, terminate on the outer hair cells and can modify their action (Warr and Guinan, 1979). More specifically, activity of these fibers causes hyperpolarisation of the outer hair cell, which alters the action of the outer hair cells on the basilar membrane, and thereby the so-called cochlear amplifier. As a consequence OAEs are reduced in amplitude (Guinan, 2006).

Geven et al. (2011) have investigated the role of the MOC in tinnitus subjects. The results were contradictory to the published literature. While others reported reduced contralateral suppression in tinnitus subjects (Chery-Croze et al., 1994a; Chery-Croze et al., 1994b; Graham and Hazell, 1994; Attias et al., 1996; Lind, 1996; Ceranic et al., 1998; Favero et al., 2006; Riga et al., 2007), both Geven et al. (2011) and Paglialonga et al. (2011) found suppression to be similar in tinnitus subjects and controls, possibly because of carefully selected subjects and controls with emissions that were detectable according to a strict signal-to-noise criterion (Geven et al., 2011). Suppression of an OAE-signal is generally measured as a decrease of the emission amplitude in different frequency bands. It might be that certain information is present in the time domain (e.g. phase shift), which is missed in this way. Because of the contradictory results of Geven et al. (2011) compared to other published literature, we felt the need for a supplementary time-frequency analysis of the measured OAE signals and their suppression.

Wavelets were chosen for this supplementary analysis of OAEs. Wavelet analysis yields both time and frequency information present in a transiently-evoked OAE signal (Wit et al., 1994; Tognola et al., 1997; Tognola et al., 1998). The result of wavelet analysis is a representation of the OAE–signal amplitude in the time-frequency plane, conserving both time information as well as frequency information. Among the different methods that can be used for time-frequency analysis, the wavelet transform seems to be the best compromise between time-frequency resolution and interference terms attenuation (Tognola et al., 1998).

Results of wavelet analysis of contralateral suppression of otoacoustic emissions in normal hearing subjects with negative otologic histories are described by Morand et al. (2000). Very recently Paglialonga et al. (2011) studied suppression of OAEs in tinnitus patients with a technique that is closely related to the wavelet analysis method that we use it in the present paper. The purpose of our study was to compare the suppression of click-evoked OAEs by contralateral acoustic stimulation in tinnitus patients and healthy controls, by wavelet analysis.

5.2 Material and Methods

5.2.1 Otoacoustic emission measurement

Click-evoked otoacoustic emissions (CEOAEs) were recorded and analyzed using the Otodynamics Ltd ILOv6 (United Kingdom). The influence of CAS on the CEOAEs was investigated in both ears of each subject. Stimuli were presented with a standard ear probe, inserted in the ear canal with a standard rubber earplug. CEOAEs were recorded in response to clicks presented at a repetition rate of 18 Hz. The peak click stimulus level was 65 dB SPL and 500 responses were averaged in order to obtain the response, for both the suppressed and unsuppressed CEOAE. To measure the effect of CAS, broadband noise was presented contralaterally at 70 dB SPL. The emissions were recorded in the standard non-linear manner. The first two milliseconds after stimulus onset of the response were suppressed.

5.2.2 Study subjects

We included (see also section 5.2.4) 26 ears (14 right, 12 left) of 20 tinnitus patients who visited our specialized out-patient tinnitus clinic (75% male, mean age 48±10 years). Median tinnitus duration was 4.0 years and ranged from 1 to 30 years. The tinnitus was

left-sided in 30%, right-sided in 20% and non-lateralized in 50% of the tinnitus patients. The cause of the tinnitus, as reported by the patients, was exposure to loud noise in 2 patients, an ear infection in 1, a systemic illness in 3, and stress in 4 patients. No cause was known for the remaining patients.

For the control group we included 37 ears (18 right, 19 left) of 26 normal hearing subjects (37% male, mean age 43±9 years). The mean audiograms for the ears of the control group and of the tinnitus patients group are shown in figure 1.

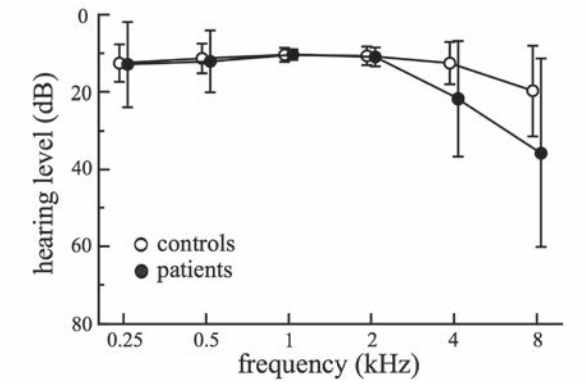

Figure 1. Mean hearing levels (± 1 SD) for the ears of the control group and of the tinnitus patients group.

5.2.3 Wavelet analysis

The wavelet analysis applied to the recorded CEOAE signals was identical to the method introduced by Wit et al. (1994), with one exception: the asymmetrical gammatone wavelet was replaced by a symmetrical gaussian wavelet (figure 2). This wavelet shape was chosen to obtain comparable resolution in the time and in the frequency domain.

The result of wavelet analysis is a 50x50 array of 2500 values for the amplitude of the CEOAE-signal in a time-frequency plane, with a linear horizontal axis from 0 to 20 ms and a linear vertical axis from 0 to 5 kHz. As an illustration figure 3 gives a simulated OAE-signal and its 3D wavelet analysis result.

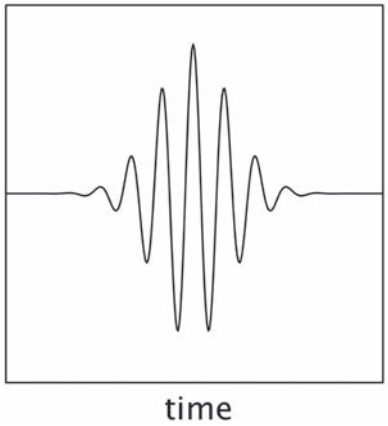

Figure 2. Wavelet with Gaussian envelope used for the analysis of OAE signals.

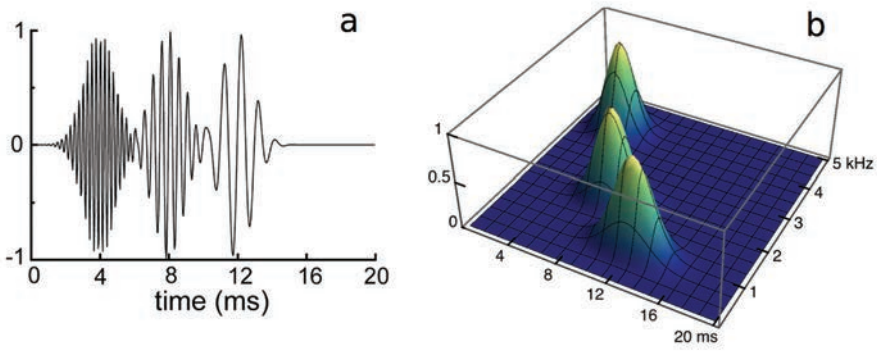

Figure 3. a: sum of three wavelets, all with the same gaussian envelope, with frequencies of 1, 2 and 4 kHz. b: result of wavelet analysis of the signal shown in panel a.

Figure 4 is an example of our wavelet analysis procedure. Figure 4a is the CEOAE of the right ear of control subject LH, measured without CAS. It is the average of the two signals a and b alternately measured by the Otodynamics ILOv6 (From the correlation between these two signals the ILOv6 software calculates the reproducibility of the OAE-signal). Figure 4b is the CEOAE of the same ear measured with CAS. Figures 4c and 4d are a 3D-representation of the result of wavelet analysis of the OAE-signal shown in figures 4a and 4b respectively. Figure 4e is the result of subtraction of the sound pressure values shown in figure 4d from the corresponding values in figure 4c. It shows the result of CAS: for this ear suppression is maximal around position (8 ms, 1 kHz) in the time-frequency plane.

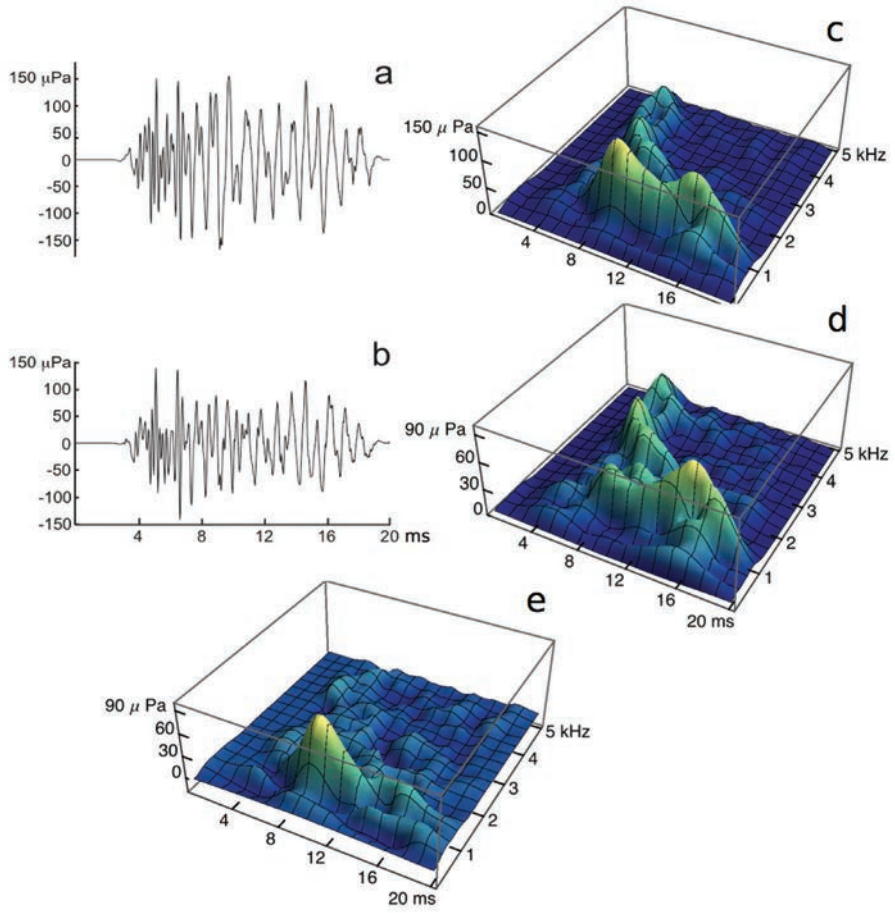

Figure 4. a: CEOAE of the right ear of control subject LH, measured without CAS;

b: CEOAE of the same ear measured with CAS; c: 3D-representation of the result of wavelet analysis of the OAE-signal in panel a; d: 3D-representation of the result of wavelet analysis of the OAE-signal in panel b; e: Difference between surfaces shown in panel c and in panel d.

As mentioned above, the ILOv6 software calculates the "reproducibility" of the OAE-signal from the correlation between two alternately measured signals *a* and *b*. If a signal *a* is constructed by adding gaussian noise n_1 to the sum *s* of three wavelets, shown in figure 3a, and a signal *b* by adding different Gaussian noise n_2 to the same sum of wavelets, Pearson's product moment correlation *r* can be calculated for signals *a* and *b*. The result is given in figure 5 (open circles) for different values for the signal to noise ratio ρ, chosen to be equal for signals *a* and *b*. Signal *a* is given by $\frac{\rho}{1+\rho}s' + \frac{1}{1+\rho}n'_1$

The same holds for *b*, if n_1 is replaced by n_2. (s' = s/RMS(s), n' = n/RMS(n); RMS = root mean square). The solid line in figure 5 is given by $r = \frac{\rho^2}{\rho^2 + \rho_0^2}$. (Correlation *r* is 0.5 for $\rho = \rho_0 = 0.756$).

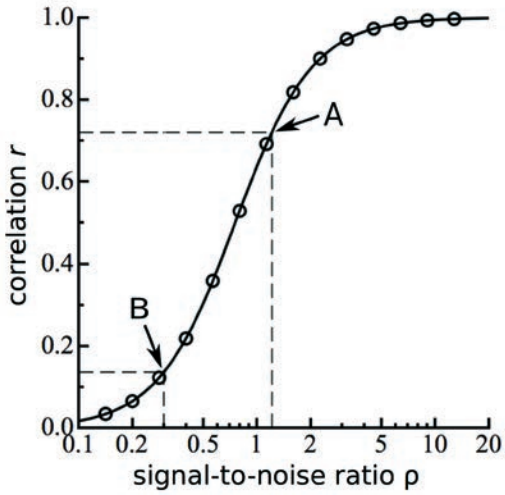

Figure 5. Relation between signal-to-noise ratio and correlation for two signals consisting of the same signal component and different gaussian noise components.

Figure 6 shows, for two values of the signal-to-noise ratio, the result of wavelet analysis of the signal (*a*+*b*)/2, in the same way as this is done for the emission signals of controls and patients. The left panel of figure 6 shows - from top to bottom - signal *a*, the 500 instantaneous values of signal *b* plotted versus the corresponding values of signal *a* and the result of wavelet analysis, for a correlation between *a* and *b* of 0.72, corresponding to the mean reproducibility of the OAE measurements without CAS in the patients group (see Results), denoted by "A" in figure 5. The right panel shows similar plots for a low value of the signal-to-noise ratio, denoted by "B" in figure 5. Although the signal cannot be distinguished from the noise in the upper figure of the right panel, the three signal components are clearly visible in the lower figure of this panel. This shows that wavelet analysis is a powerful method to separate a signal from broadband noise, if the signal is confined to a restricted area of the time-frequency plane, and thus well suited to detect small changes in OAE responses.

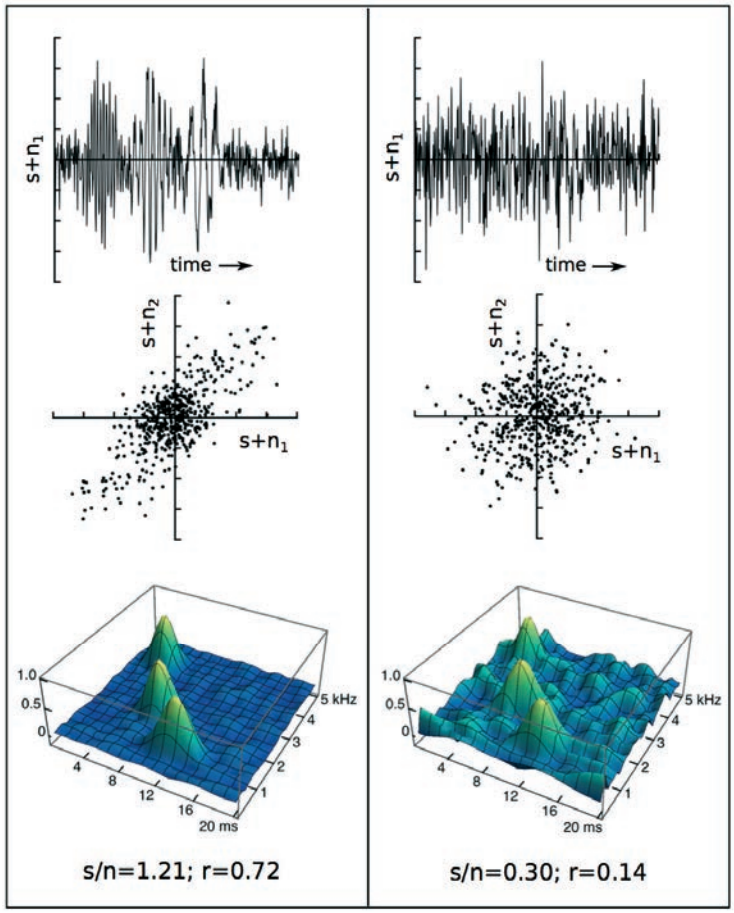

Figure 6. From top to bottom: signal a(t), b(t) versus a(t), wavelet analysis result for (a+b)/2, for two values of signal-to-noise ratio s/n. (See text for a definition of the signals).

5.2.4 Inclusion criteria

1. The whole-wave reproducibility, as calculated for the OAE-signal by the Otodynamics Ltd ILOv6 software, had to be 50% or better.

2. Maximum suppression of the OAE-signal by CAS had to be in the time-frequency area specified as follows: in this area the wavelet analysis result of the unsuppressed OAE-signal for that ear has an amplitude of 50% or more of the peak value. This makes sure that CAS suppressed the OAE-signal and that the difference between the unsuppressed and the suppressed waveform is not a difference in noise contamination. The criterion

is illustrated in figure 7. This figure is a contour plot for the result of wavelet analysis of the OAE-signal of the left ear of control subject EW. In the white areas the amplitude of the OAE-signal is 50% or more of the maximum amplitude. For this ear suppression by CAS is maximal at the time-frequency coordinates of the star (so this ear was included).

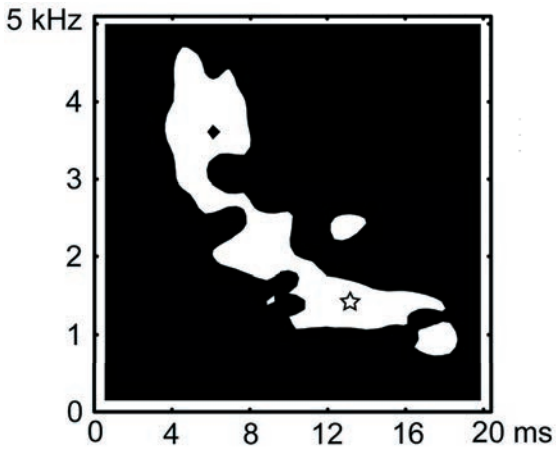

Figure 7. Contour plot for the result of wavelet analysis of the OAE-signal of the left ear of control subject EW, unsuppressed by CAS. At the time-frequency coordinates of the star suppression by CAS was maximal for this ear.

5.2.5 Exclusion criterion

Ears for which the OAE-signal amplitudes above the noise floor were only seen in the upper right quadrant of the time-frequency plane were excluded. It is very unlikely that an ear produces CEOAEs with high frequencies at long delays.

5.2.6 Statistics

Differences between means will be considered to be significant for $p < 0.05$.

5.3 Results

5.3.1 Reproducibility

The whole-wave reproducibility (calculated as 100 x Pearson's correlation between the two signals alternately measured by the Otodynamics Ltd ILOv6) without CAS is 74±14% for the control group and 72±20% for the patients.

5.3.2 Wavelet analysis results

Figure 8 shows the average result in 3D-representation of the wavelet analysis of the CEOAEs measured without CAS of all the included ears for both the patients and the control group. (The average result is the sum of the 50x50 arrays – as mentioned in the wavelet analysis section - for all included ears in a group, divided by the number of ears in that group). The figure shows the characteristic property for CEOAE's: high frequency components are measured earlier in time than low frequency components.

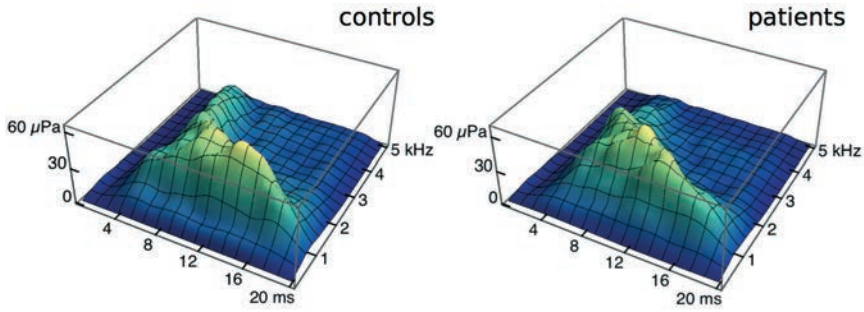

Figure 8. 3D-representation of the average result of wavelet analysis of the OAEs measured without CAS, for all included ears of the control group (n=37) and of the tinnitus patient group (n=26).

Figure 9 shows contour plots for the same data as plotted in figure 8. The number of contours in this figure is 4, separating 5 amplitude ranges in the time-frequency plane, between zero and the maximum amplitude value, along a linear scale.

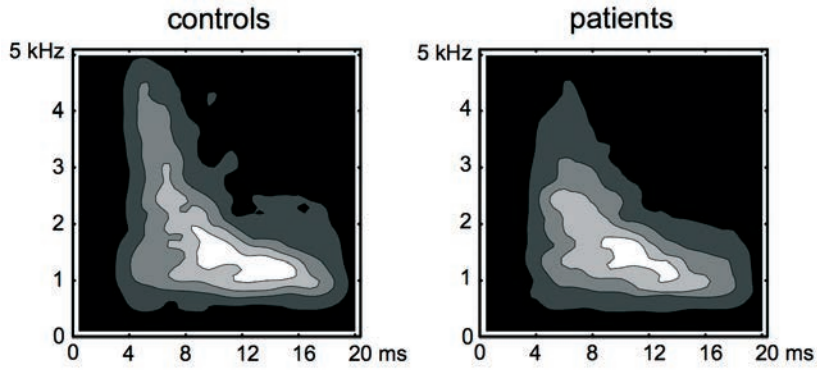

Figure 9. Contour plots for the average result of wavelet analysis of the OAEs measured without CAS, for all included ears of the control group and of the tinnitus patient group. (White = highest amplitudes; black = lowest amplitudes).

The maximum average OAE-level in figures 8 and 9 is 65 µPa at position (13.6 ms, 1.2 kHz) for the controls and 68 µPa at position (10.4 ms, 1.5 kHz) for the patients. At 4kHz the maximum level is 31.7 µPa (at 5.6 ms) for the controls. For the patients the corresponding value is 18.7 µPa (at 6.0 ms), which is approximately 5 dB below the level for the controls.

The procedure to obtain the normalized suppression by CAS for each subject is illustrated in figure 10. Figure 10a is the "raw" suppression signal (shown in figure 4e for subject LH). Because no CEOAE-components are present in the upper right quarter of the time-frequency plane (high frequencies with long delays; bordered by the "fence" in figure 10a) signal values in this area must be noise. Only sound pressure values that are significantly above the noise floor are the result of CAS. The mean (m) and the standard deviation (s) for the 625 signal values in the bordered area are calculated and the 3D-surface in figure a is corrected as follows: if a signal value is smaller than m+3d it is replaced by zero. The result is shown in figure 10b. Next all coordinates in the time-frequency plane for which the corrected suppression value is at least $10^{-0.5}$ (- 10 dB) times the maximum suppression value are determined. These coordinates lie within the black areas in figure 10c. Then the corrected suppression values, as shown in figure 10b, are all divided by a sound pressure value p_0, which is a measure for the unsuppressed CEOAE-signal. The result is the normalized suppression shown in figure 10d. (The only difference between figures 10b and 10d is the vertical scale). Sound pressure value p_0 is the average of the sound pressure values for the unsuppressed CEOAE-signal (shown in figure 4a for subject LH), for which the coordinates lie within the black areas in figure 10c.

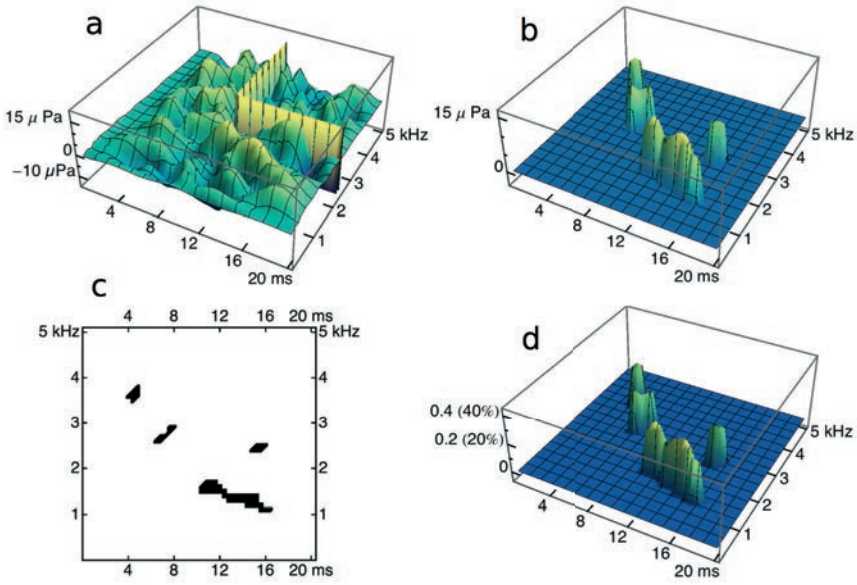

Figure 10. Illustration of the procedure to obtain the normalized suppression for the left ear of control subject EW (see figure 7), as described in the text.

The 3D-representation of the averaged normalized suppression for the control group (n=37) is shown in the upper left panel and for the patients group (n=26) in the upper right panel of figure 11. The lower panels give the difference in suppression for the two groups. In figure 11 normalized suppression is expressed in dB, for a better comparison with suppression values given by others. For this purpose normalized suppression in dB (y) was calculated with $y = -20.^{10}\log[(100-x)/100]$, in which x is the averaged normalized suppression in percent (%). For small values of x the relation between x and y is (almost) linear.

The maximum average normalized suppression for the control group (figure 11, upper panels) is 1.87 dB at the position {11.2 ms, 1.0 kHz} in the time-frequency plane. For the patients group the corresponding values are 2.33 dB at {13.2 ms, 0.9 kHz}. Averaging over that area of the time-frequency plane where the averaged normalized suppression is not zero for the controls or for the patients (figure 11, upper panels) gives 0.39 dB for the controls and 0.42 dB for the patients as the average height of the "mountain landscape". The maximum differences in averaged normalized suppression (figure 11, lower panels) are 1.08 dB at the position {6.4 ms, 1.8 kHz} and 1.45 dB at position {13.2 ms, 0.9 kHz}. At the first position the suppression in the control group is larger than that in the patients group. At the second position the suppression in the patients group is largest.

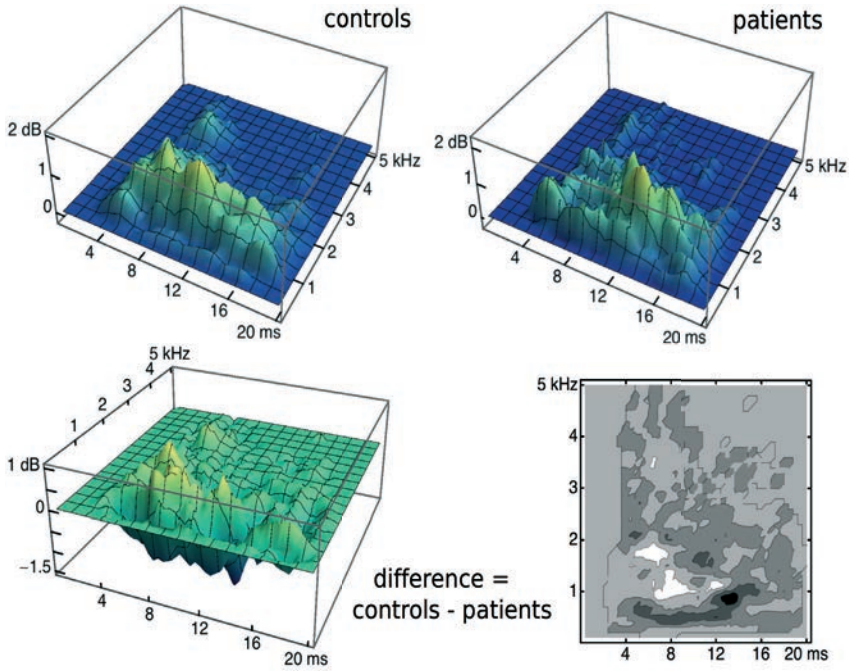

Figure 11. Upper panels: 3D-representation of the average normalized suppression for all included ears of the control group (n=37) and of the tinnitus patient group (n=26). Lower panels: Difference of the surfaces shown in the upper panels in 3D and as a contour plot.

5.3.3 Statistical analysis

The individual suppression patterns are rather different (see e.g. figures 4e and 10a), leading to an irregular 3D-representation of averaged normalized suppression for the controls and the patients and their difference (figure 11). Therefore, to investigate the significance of this difference, the mean for 100 suppression values was calculated for ten 4 ms by 1kHz squares in the time-frequency plane. These ten squares cover the area of the time-frequency plane where the averaged normalized suppression is not zero for the controls or for the patients (see figure 12a).

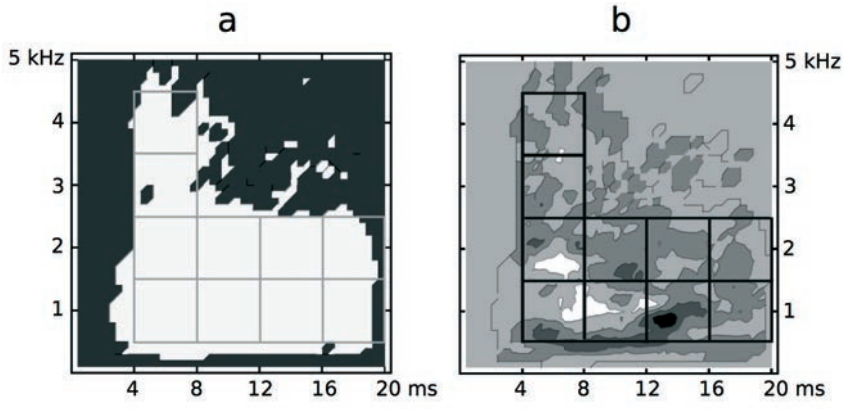

Figure 12. a: White area: part of the time-frequency plane where the averaged normalized suppression is not zero for the controls or for the patients. Grey lines: borders of ten 4 ms by 1 kHz squares used in the statistical analysis. b: Contour plot for the difference in averaged normalized suppression between controls and patients, as shown in figure 11. Black lines border the same square areas as in a.

Also calculated from the individual suppression patterns for the controls and the patients were the corresponding standard deviations. The results are shown in figures 13 and 14.

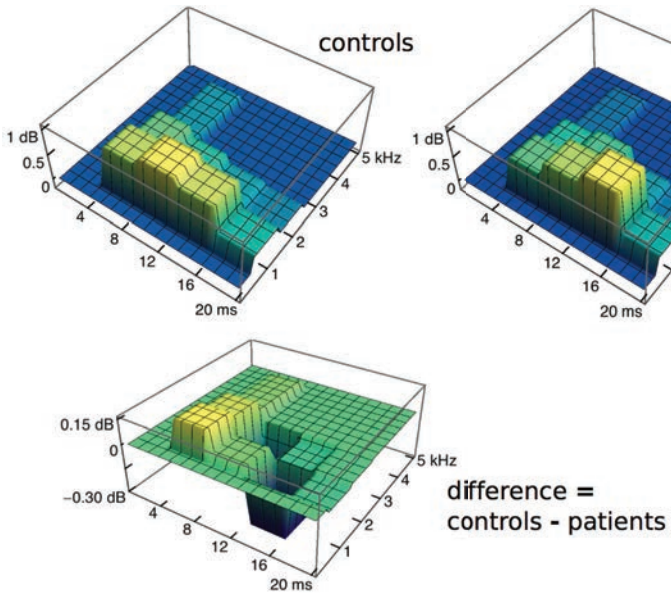

Figure 13. Upper panels: 3D-representation for ten square areas of the time-frequency plane of the average normalized suppression for all included ears of the control group (n=37) and of the tinnitus patient group (n=26). Lower panel: Difference of the surfaces shown in the upper panels.

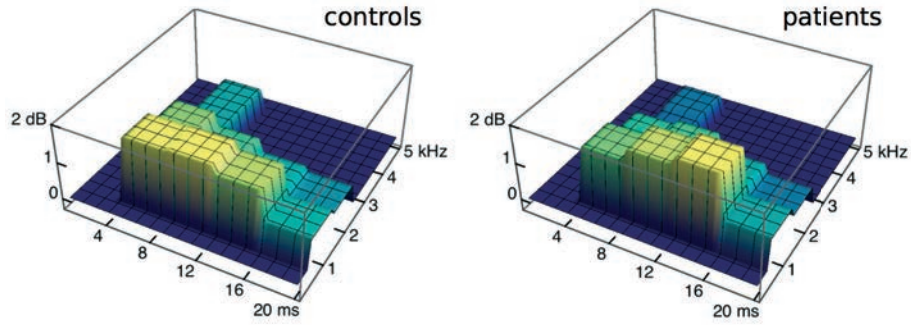

Figure 14. 3D-representation for ten square areas of the time-frequency plane of the standard deviation in normalized suppression for all included ears of the control group (n=37) and of the tinnitus patient group (n=26).

Table 1 summarizes the means and corresponding standard deviations for the ten squares shown in figures 13 and 14.

Table 1. Means and standard deviations for the ten square areas of 4 ms by 1 kHz in figures 13 and 14. The first two columns give the position of the squares in the time-frequency plane. mc = mean controls (nc = 37), sc = standard deviation controls, mp = mean patients (np =26), sp = standard deviation patients, es=effect size.

Δt(ms)	Δf(kHz)	mc(dB)	sc(dB)	mp(dB)	sp(dB)	mc-mp(dB)	es	tscore
4-8	0.5-1.5	0.77	1.67	0.60	1.44	0.17	0.11	0.41
8-12	0.5-1.5	0.93	1.71	0.89	1.76	0.04	0.02	0.10
12-16	0.5-1.5	0.80	1.50	1.10	1.94	-0.30	-0.18	-0.70
16-20	0.5-1.5	0.30	0.72	0.33	0.76	-0.03	-0.04	-0.16
4-8	1.5-2.5	0.53	1.35	0.41	1.04	0.12	0.10	0.40
8-12	1.5-2.5	0.35	1.02	0.53	1.17	-0.18	-0.17	-0.66
12-16	1.5-2.5	0.20	0.68	0.23	0.76	-0.04	-0.05	-0.20
16-20	1.5-2.5	0.12	0.54	0.14	0.50	-0.01	-0.02	-0.10
4-8	2.5-3.5	0.17	0.65	0.11	0.41	0.05	0.10	0.37
4-8	3.5-4.5	0.17	0.66	0.12	0.48	0.05	0.09	0.35

The last column in Table 1 gives the t-scores for the differences between the means for controls and for patients. These scores are calculated for the situation of unequal sample sizes and equal variances (the score is negative if suppression in the patient ears is larger than suppression in the control ears). Differences between mean normalized suppressions are defined as significant for a confidence level of 95% (p = 0.05) or better. For the number of degrees of freedom $n_c+m_p-2 = 61$ this is true for t-values outside the interval (-2.00, 2.00). None of the t-scores in the last column of Table 1 fulfils this criterion. So we cannot conclude for any of the ten square areas in figure 12 that the average normalized suppression for the tinnitus patients differs significantly from that for the control group.

5.4 Discussion

At 4 kHz the maximum average CEOAE-level in the measurements without suppression by CAS is approximately 5 dB weaker in the patients ears than in the control ears (figures 8 and 9). This is most likely due to high frequency hearing losses in the patient group (see figure 1): Prieve et al. (1993) investigated the relation between hearing loss and CEOAE-level in 113 subjects in 4 one octave wide frequency bands. For ears with a hearing loss at 4 kHz larger than 20 dB the CEOAE-level in the 4 kHz band was 5-15 dB weaker than this level for normal hearing ears (Prieve et al., 1993; figure 5).

As can be seen in figures 11 and 13 suppression of CEOAEs by CAS with broadband noise is maximal for frequencies around 1 kHz. This is in accordance with the finding of Morand et al. (2000), who state that the effect of CAS on emissions is larger at lower frequencies. In the lower frequency range (0.25 – 2 kHz) hearing is normal for both the control and the patients group (figure 1). Maximum average suppression by CAS is of the order of 1 dB (figures 11 and 13 and Table 1). Although different authors use different definitions for suppression this number corresponds well with values given by others (Collet et al., 1990; Giraud et al., 1995; Hood et al., 1996; Favero et al., 2006; Sun, 2008).

The present study confirms earlier results (Geven et al., 2011) and the study of Pagliolonga et al. (2011) that suppression of CEOAEs by CAS is measurable in tinnitus patients. Also distortion product otoacoustic emissions (DPOAEs) are suppressed in tinnitus ears by CAS with white noise (Riga et al., 2007). For our subjects (both controls and tinnitus patients) average normalized suppression is significant for most of the ten square areas of 4 ms by 1 kHz in figures 13 and 14, as shown in Table 2. This is in clear contrast with the result by Attias et al. (1996) who found enhancement of CEOAEs instead of suppression by CAS in tinnitus sufferers. The finding in the study of Geven et al. (2011) that a small difference exists between suppression measured in the right ear in tinnitus patients and normal controls for 2 half octave wide frequency bands, centered around 2.0 and 2.8 kHz, differs from the result in the present study. This difference may be caused by a different number of included ears, due to different inclusion criteria. In the earlier study 57 ears were included in the control group and 44 in the tinnitus group. In the present study these numbers are 37 and 26 respectively. Another reason may be the difference in quantification of suppression. In our earlier study suppression is the difference in dB between the OAE measured without and with CAS. Levels of the measured OAEs are – per frequency band – given by the Otodynamics equipment. In the present study normalized suppression is calculated as explained in figure 10 in section 5.3.2.

Table 2. Means and standard deviations for the ten square areas of 4 ms by 1 kHz in figures 13 and 14. The first two columns give the position of the squares in the time-frequency plane. mc = mean controls (nc = 37), sec = standard error controls, mp = mean patients (np =26), sep = standard error patients, pc and pp = probability that suppression is zero (or negative) resp. for controls and patients. For the values marked with an asterisk suppression does not significantly (at the 5% level) differ from zero.

Δt(ms)	Δf(kHz)	mc(dB)	sec(dB)	pc(%)	mp(dB)	sep(dB)	pp(%)
4-8	0.5-1.5	0.77	0.27	0.25	0.60	0.28	1.68
8-12	0.5-1.5	0.93	0.28	0.05	0.89	0.34	0.50
12-16	0.5-1.5	0.80	0.25	0.06	1.10	0.38	0.19
16-20	0.5-1.5	0.30	0.12	0.56	0.33	0.15	1.34
4-8	1.5-2.5	0.53	0.22	0.85	0.41	0.20	2.22
8-12	1.5-2.5	0.35	0.17	1.84	0.53	0.23	1.04
12-16	1.5-2.5	0.20	0.11	3.68	0.23	0.15	6.14*
16-20	1.5-2.5	0.12	0.09	8.82*	0.14	0.10	7.67*
4-8	2.5-3.5	0.17	0.11	5.58*	0.11	0.08	8.57*
4-8	3.5-4.5	0.17	0.11	5.85*	0.12	0.09	10.1*

The differences between the mean normalized suppressions for controls and patients, as given in the 7-th column ("m_c-m_p") of Table 1, are small (maximum 0.3 dB) compared to the standard deviations for controls and patients, as shown in columns 4 and 6 of the same table. This leads to small values for the "effect size" or "Cohen's d" (Cohen, 1988), given by: $d = \frac{m_c - m_p}{s}$, in which s is the "pooled standard deviation", given by: $s = \sqrt{\frac{(n_c-1)s_c^2 + (n_p-1)s_p^2}{n_c+n_p}}$; ($n_p$ and n_c are the numbers of controls and patients ears respectively).

The reason for the relatively large standard deviations in normalized suppression is most likely the large difference in time-frequency representation of OAEs from different ears, leading to different suppression patterns. This difference is illustrated in figure 15 for 6 control ears. The relation between t-score t and effect size d is given by: $t = \frac{\sqrt{(n_c+n_p-2)n_c n_p}}{n_c+n_p} \cdot d$.

For $n_c = 37$ and $n_p = 26$ this gives $t = 3.85d$. Statistical power increases if the effect size increases and/or the number of subjects. A difference of 0.3 dB between suppression in patients and in controls (Table 1, third row of numbers) would –for the same values for m_c, s_c, m_p and s_p as shown – be significant at the 95% level ($t > 2.00$) if the number of subjects would have been larger than 268 in both groups (we suppose that $n_c = n_p$). This is about ten times the number of subjects in the present study.

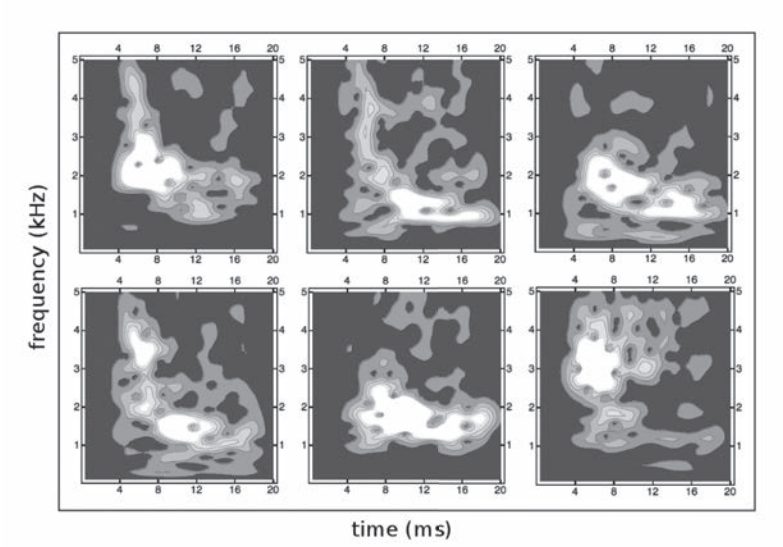

Figure 15. Contour plots (4 contours) for the result of wavelet analysis of OAEs for 6 control ears, measured without CAS. (white = highest amplitudes; black = lowest amplitudes).

CEOAEs were measured in the standard (clinically most often used) non-linear mode, eliminating signal components that are proportional to stimulus level. It is conceivable that suppression by CAS of CEOAEs measured in the linear stimulation mode differs from suppression measured in the non-linear mode. De Ceulaer et al. (2001) found for measurements in the non-linear mode that suppression by contralateral white noise is of the same order of magnitude as suppression measured in the linear mode, so possible differences – if present – are small.

The CAS activator in the present study was broadband noise with a level of 70 dB SPL. Sun (2008) states (for distortion product otoacoustic emissions) that contribution of the middle ear muscle (MEM) reflex is minimal, if any, to the change of DPOAE-level if CAS is applied at a level below the acoustic reflex threshold (ART) for broadband noise as the activator. In his study with normal hearing young adults ART ranged from 70 to 100 dB SPL (median 85 dB SPL). This author used a clinical middle ear muscle analyser to determine the reflex threshold. When comparing MEM-reflex thresholds for a 4000 Hz activator tone measured with a clinical system or with an experimental wideband reflectance and admittance system, Feeney et al. (2004) found approximately 3 dB lower thresholds with the second method compared to the clinical method. When using contralateral broadband noise as the activator and their sensitive wideband

reflectance and admittance measuring method to determine MEM-reflex thresholds, Feeney and Keefe (2001, Table 2) found an average threshold value of 62 dB SPL. So it is not unlikely that part of the suppression, as we measured it, is the result of middle ear muscle activity, instead of an effect on the inner ear, where OAEs are generated.

The role of the entire efferent auditory system in the origin or maintenance of tinnitus is still speculative. The extensiveness of the efferent auditory pathways (Winer, 2006) makes, among other things, that many questions remain unanswered to this date. And for ethical and legal reasons many of these questions are currently not open for investigation in humans. In this paper we have described our research concerning only a small part of the efferent auditory system, from the brainstem to the outer hair cells in the cochlea (Warr and Guinan, 1979). If the efferent auditory system is indeed involved in the physiology or maintenance of tinnitus, it will be the part that is central to the medial olive of the brainstem.

Recently Mulders et al. (2010) suggested that focal and specific pharmacological manipulation of the olivocochlear system could offer therapeutic possibilities for the treatment of tinnitus. Their suggestion is based on the results of electrical stimulation of olivocochlear axons in anesthetized guinea pigs. After exposure to loud sound and subsequent recovery these guinea pigs showed hyperactivity in the central auditory pathways, which could be diminished by direct stimulation of the olivocochlear axons. So potentially, stimulation of a normal functioning MOC in tinnitus patients could provide relief from the tinnitus. Testing for a normal functioning auditory efferent system in tinnitus patients is an essential step for exploring this new potential therapy.

In summary: we have shown that suppression by contralateral acoustic stimulation of click-evoked otoacoustic emissions in tinnitus patients is to a large extent comparable with that in subjects without tinnitus. So, we found no indication for abnormal functioning of the medial olivocochlear system in tinnitus patients.

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Chapter 6

The effect of TMS on otoacoustic emissions: a pilot study probing the efferent auditory system

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6.1 Introduction

Otoacoustic emissions (OAEs) are sounds of cochlear origin, caused by the outer hair cell motion in the cochlea (Kemp, 1978). Their amplitude can be influenced by contralateral acoustic stimulation (CAS)(Mott et al., 1989). Part of the efferent auditory system, called the medial olivocochlear system (MOC), is responsible for this amplitude change after CAS (Guinan, 2006). The MOC originates in the medial part of the superior olivary complex, reaching the contralateral cochlea through the vestibulocochlear nerve. The efferent neurons of the MOC system contact outer hair cells in the cochlea, which generate OAEs. Usually, CAS gives a reduction in OAE amplitude, which is called contralateral suppression.

The efferent auditory system runs from cortex to cochlea, with connections to both auditory and non-auditory brain areas (Winer, 2006). Most functional knowledge on this pathway comes from animal research. Recently the existence of functional corticofugal projections in humans was demonstrated for the first time (Perrot et al., 2006). In their study direct electrical stimulation of the auditory cortex with deep brain electrodes resulted in decrease of the amplitude of the contralateral OAEs. Electrical stimulation of non-auditory areas did not cause a decrease in amplitude of the OAEs (Perrot et al, 2006). These results demonstrated the functional efferent connection between the auditory cortex and the cochlea in humans.

Since the efferent auditory pathway can be stimulated with direct electrical stimulation of the auditory cortex (Perrot et al. 2006), possibly indirect transcranial magnetic stimulation (TMS) can do the same. TMS is a relative new technique that electrically stimulates the brain through the intact skull and scalp. It uses a focussed magnetic field that changes rapidly. This induces an electrical current, effecting cortical and subcortical tissue and activity (Ridding and Rothwell, 2007). Stimulation with low-frequency repetitive TMS (1 Hz) resulted in a prolonged decrease in cortical excitability in healthy humans (Chen et al., 1997; Hoffman and Cavus, 2002; Ridding and Rothwell, 2007). Long-lasting changes in neural activity in the cortex have been demonstrated in gerbils with the maximum duration of 24 hours, outlasting the duration of the stimulation by many hours (Wang et al., 1996). TMS has been used successfully to treat several different hyperexcitability disorders, for example auditory hallucinations in schizophrenia (Hoffman and Cavus, 2002; Aleman et al., 2007).

If TMS electrically stimulates the primary auditory cortex, we expect that this can be demonstrated by measuring changes in the amplitude of the contralateral otoacoustic emissions, comparable to the study by Perrot et al. (2006). The reduction in the amplitude of OAEs after TMS will then be an objective tool to demonstrate the activation of efferent connections from the cortex to the cochlea. To our knowledge, no study has been published that measured the effect of TMS on the amplitude of OAEs. Therefore, we first aimed to assess the effect of TMS on OAEs of healthy, normal hearing subjects. Obviously, the exact TMS frequency best suited for decreasing the amplitude of the OAEs has also not been studied. In this study both low-frequency stimulation (1 Hz) as well as high-frequency (10 Hz) TMS were used for stimulation. Our hypothesis is that TMS stimulation of the auditory cortex activates the efferent auditory system down to the outer hair cells in the cochlea. The hypothesis is confirmed if TMS stimulation changes the amplitude of the contralateral click-evoked OAEs. If the effect of TMS stimulation is similar to that of direct electrical stimulation (Perrot et al., 2006), OAE amplitudes are expected to decrease.

6.2 Experiment I: TMS of the primary auditory cortex and contralateral otoacoustic emissions.

6.2.1 Methods

6.2.1.1 Study subjects

We included fifteen healthy, normal hearing subjects for this study (mean age 24 years, range 20-28, SD 2.4 years, 5 male subjects). All subjects were without medical or otologic history. All subjects were screened with click-evoked otoacoustic emissions (CEOAEs) and contralateral suppression in both ears. Subjects were included when whole signal reproducibility of the OAE signal was over 70% with a minimum of 6 dB SPL signal-to-noise ratio (SNR) in at least 3 half-octave frequency bands. Also, the inclusion of participants was in accordance with international safety guidelines of rTMS (Rossi et al., 2007). Written informed consent was obtained from all the subjects before the start of the study. The study was approved by the medical ethics committee of the University Medical Center Groningen and in accordance with the declaration of Helsinki.

6.2.1.2 Study Protocol

The design of the study was an exploratory placebo-controlled intervention study in a Latin-square cross-over design (see Figure 1). After inclusion, the subjects were

divided at random in three groups. Each group started at a frequency of 1 Hz, 10 Hz or placebo rTMS stimulation. Before and after each TMS session, OAEs were measured. The OAE-measurements followed the TMS-session as close in time as possible (within 30 seconds). All subjects had a minimum of 1 week between the different TMS sessions. Each subject completed the study protocol blinded to the stimulation condition.

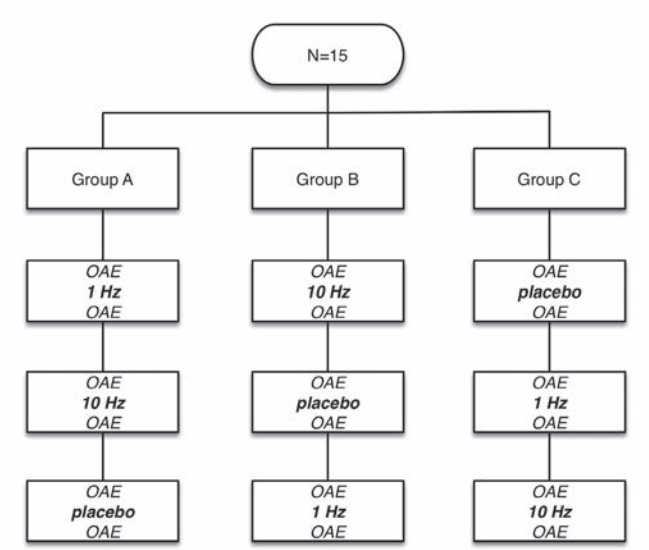

Figure 1. Flowchart of the cross-over study design. Study subjects were randomly assigned to one of the groups. After randomisation each panel represents one experimental session. A minimum of 1 week separated each session. Before and after each rTMS session, OAEs were measured.

6.2.1.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

Focal rTMS was applied using MedTronic MagPro connected to a figure-of-eight stimulation coil, type Cool-B65. To position the coil over the left auditory cortex we used the estimated position based on the 10-20 electroencephalography (EEG) system, as suggested by Langguth et al. (2006). For this system we used caps that were fitted to the individual head size. On these caps of the 10-20 EEG system, we marked the position of the left Heschl's Gyrus where the primary auditory cortex is located (Langguth et al., 2006).

Before TMS, we determined the motor threshold (MT) for each subject. For this determination, the coil was applied over the primary motor cortex. The MT was defined as the amplitude for which 5 out of 10 consecutive single pulse TMS elicited an observable contraction of the thenar muscle, quantifying the individual cortical

excitability. The three stimulation sessions were performed with the individual 100% MT. All subjects wore disposable foam earplugs during the rTMS session to prevent the influence of the noise of the TMS coil on the OAEs.

The 1-Hz rTMS session was carried out for 15 minutes with the intensity of 100% MT over the left auditory cortex. For the 10-Hz rTMS we used interval trains. This was done with 36 trains of 25 stimuli for every 30 seconds up to 900 stimuli (Khedr et al., 2008). During placebo stimulation the same coil was used as during active stimulation. With placebo stimulation, the coil was tilted 90° two-wing off the head. Placebo stimulation was at 1 Hz for 15 minutes with a total amount of 900 stimuli.

6.2.1.4 Otoacoustic emissions

CEOAEs were recorded and analyzed using the Otodynamics Ltd ILOv6 (United Kingdom). We performed standard CEOAE measurements, with a peak stimulus level of 70 dB SPL. For each ear, the responses of at least 300 sets of 4x2 stimuli (i.e. three in phase, one in opposite phase with triple amplitude; in buffers A and B) were averaged in about 70 seconds, in order to obtain the CEOAE signal. Emissions were recorded using the standard non-linear stimulation method. The artefact rejection level was 50 dB SPL. The ILO device calculated the total broadband CEOAE response and noise level, as well as in five half-octave frequency bands centered at 1.0, 1.4, 2.0, 2.8 and 4.0 kHz. The change in amplitude was calculated by subtracting the CEOAE signal amplitude after rTMS from the signal amplitude before rTMS, for each frequency band and the total response. Amplitude change in a particular frequency band was only considered if the CEOAE signal met 2 criteria: (1) the whole-wave reproducibility for the CEOAE was 70% or better, and (2) the signal-to-noise ratio (SNR) in the band was 3 dB or higher, before and after the rTMS.

6.2.1.5 Data Analysis

Statistical analysis of the amplitude change and the different stimulation paradigms was performed in SPSS software (SPSS 16.0 Inc. Chicago). Non-parametric testing was performed when necessary with the Wilcoxon Signed Ranks Test, the Friedman test for multiple related samples or Kruskal-Wallis test for multiple unrelated samples.

6.2.2 Results experiment I

All subjects tolerated the TMS procedure without developing seizures or any other serious side effects. Three subjects reported mild headache after the 10-Hz stimulation

procedure, without the need for medication. The individual MT of stimulation ranged between 40% and 56% of the maximal stimulator output (mean 50%, SD 4.7). The intensity of the stimuli for CEOAE ranged from 67.6 to 75.2 dB SPL, with a mean of 69.3 dB SPL (SD 1.1). The reproducibility of the CEOAE response ranged from 75% to 98%, with a mean of 92%, (SD 6.0).

6.2.2.1 Effects of rTMS

Figure 2 shows the results of the OAE measurements, before and after the three stimulations, for the total response (panels a, c, and e) as well as for the different frequency bands (panels b, d, and f). Overall, with 1-Hz rTMS the total OAE amplitude increased with a mean of 0.70 (SD 0.78) dB SPL after the TMS treatment. This difference was significant ($p = 0.008$, Wilxocon signed ranks test). After the 10-Hz stimulation the total OAE amplitude increased with a mean of 0.80 dB SPL (SD 1.06, $p = 0.01$, Wilcoxon signed ranks test). Interestingly, after placebo stimulation the total OAE amplitude increased as well (mean 1.09 dB, SD 1.84, $p = 0.007$, Wilcoxon signed ranks test). The OAE amplitude for the 3 different conditions stratified in the five half-octave bands is shown in figure 2 as well, with the number of included ears (SNR ≥ 3 dB) per half-octave frequency band in Table 1. Statistical significant differences are marked with an asterisk ($p<0.05$, Wilcoxon signed ranks).

6.2.2.2 Effects of rTMS frequency

There was no significant difference in the amount of change in OAE amplitude between 1 Hz, 10 Hz or placebo ($p>0.05$, Friedman test), when measured for the total response and the 5 half-octave frequency bands separately. This is shown in figure 3. There was no significant difference in amount of change in OAE amplitude between group A, B or C ($p>0.05$, Kruskal-Wallis test).

Table 1. Number of included ears per frequency band, with a SNR of 3 dB or higher

Frequency (kHz)	Number of included ears		
	1 Hz	10 Hz	Placebo
1.0	12	13	11
1.4	14	14	15
2.0	15	15	15
2.8	15	15	14
4.0	12	10	9

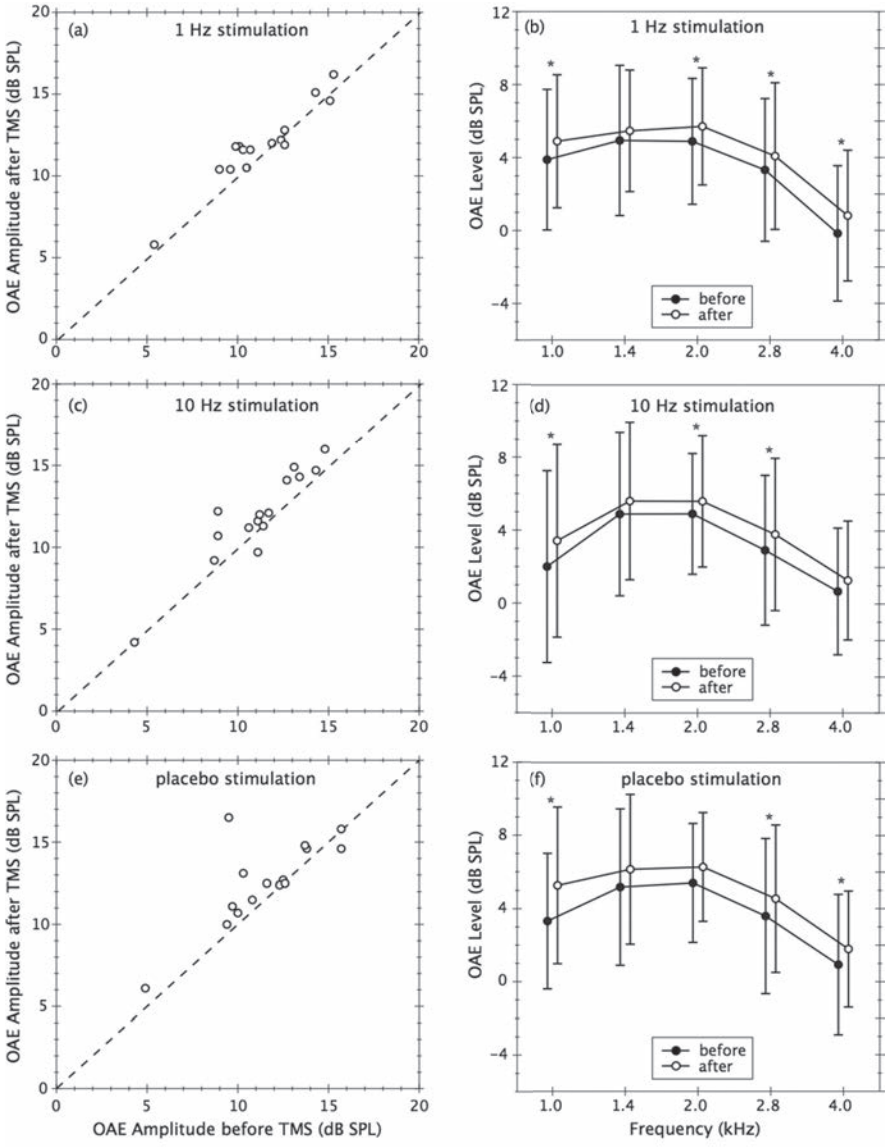

Fig. 2. (a): Total OAE amplitude before and after 1 Hz rTMS. Every point represents 1 subject; (b): The mean amplitude (and standard deviation) of the CEOAEs before and after 1 Hz rTMS stimulation. Only frequency bands with a SNR of 3 dB or more before and after stimulation were included (see Table 1). Differences in CEOAE amplitude were statistically significant for frequencies marked by an asterisk. Panels (c) and (d): Same for 10-Hz rTMS stimulation; Panels (e) and (f): Same for placebo rTMS stimulation.

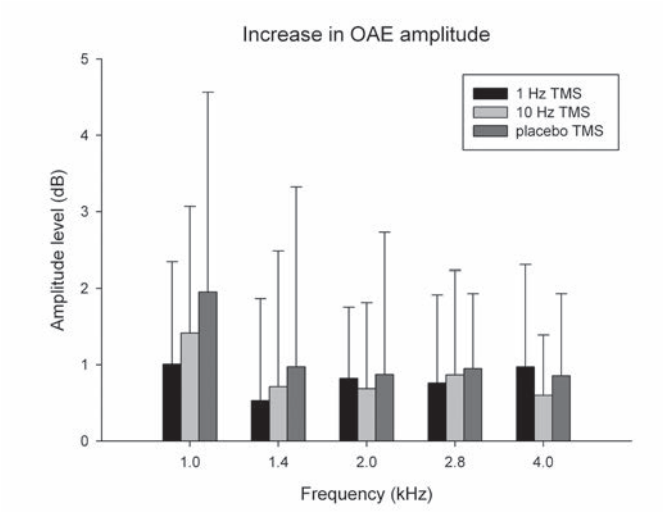

Fig. 3. The amount of change (mean and standard deviation) in the OAE amplitude, calculated as the difference in amplitude between the CEOAE after and before the various types of TMS.

6.3 Experiment II: Otoacoustic emissions and earplugs.

6.3.1 Rationale and Methods

In experiment I we aimed to explore if rTMS could influence the amplitude of CEOAEs via stimulation of the efferent auditory pathway. We measured a small and significant increase in amplitude in all conditions, including the placebo condition. This suggested that another factor than the rTMS was involved in the changes in the OAE amplitude. During the rTMS stimulation, all subjects wore earplugs to prevent the rTMS clicking sounds to influence the CEOAE amplitudes. The loud TMS sound could temporarily change hearing thresholds or otoacoustic emission amplitudes (e.g. Le Prell et al., 2012). Therefore, the subjects wore bilateral earplugs to minimise the influence of sound during experiment I. But, the occlusion of the ear canal with earplugs could have been the decisive factor influencing the CEOAEs in experiment I. To test this hypothesis, we measured CEOAEs again in 12 of the 15 originally included subjects. The measurement of the CEOAEs was exactly identical to the initial method, but no rTMS was performed. All 12 subjects wore earplugs in both ears for 15 minutes. The CEOAEs before and after the 15 minutes of wearing earplugs was compared. In addition we measured OAEs in 5 subjects of these 12, but this time before and after 15 minutes of waiting in a quiet room without wearing earplugs; so no intervention was performed.

6.3.2 Results experiment II

After wearing earplugs, the total OAE amplitude increased with a mean of 0.93 dB SPL (SD 0.8) for the right and left ear. This difference was significant ($p < 0.001$, Wilcoxon signed ranks test). Of the 5 subjects in which CEOAEs were additionally measured before and after 15 minutes without wearing earplugs, the mean OAE amplitude decreased 0.10 dB SPL (SD 0.5) for the left and right ear. This difference was not significant ($p>0.05$, Wilcoxon signed ranks test). Thus, CEOAE amplitude increased after wearing earplugs and remained unchanged after 15 minutes without any intervention. The results for the total OAE response (panels a and c), as well as for the different frequency bands (panels b and d) in the condition with an earplug and without any intervention, are shown in figure 4.

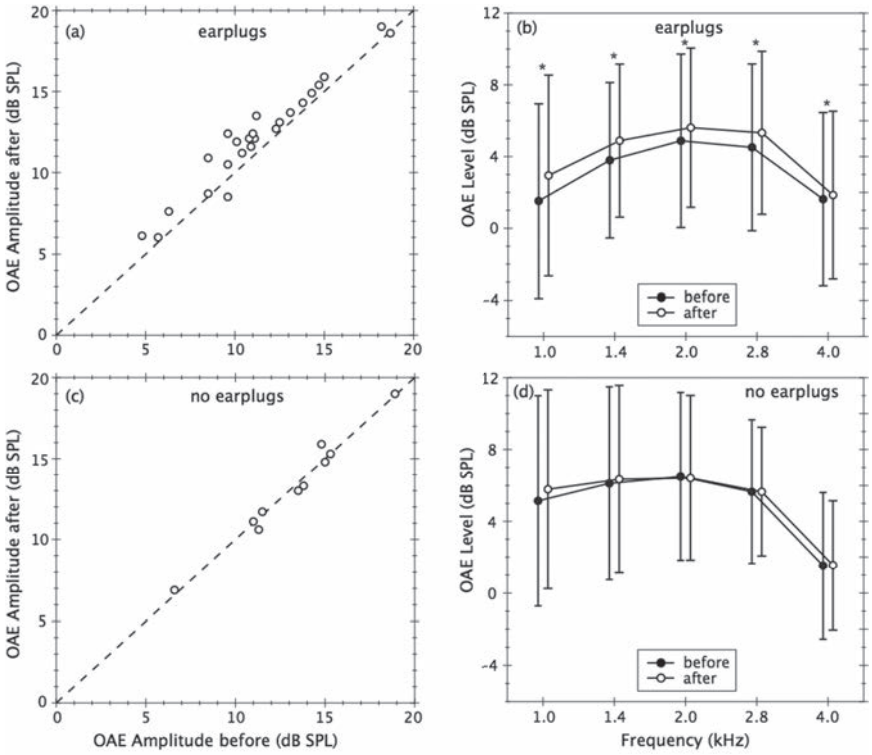

Fig. 4. (a): Total OAE amplitude before and after 15 minutes of wearing an earplug in both ears, results for right and left ear of 12 subjects. Every point represents 1 ear of 1 subject; (b): The mean amplitude (and standard deviation) of the CEOAEs before and after 15 minutes of wearing an earplug. Differences in CEOAE amplitude were statistically significant for frequencies marked by an asterisk. Panel (c) and (d): Same for 15 minutes without intervention for 5 subjects.

6.4 Discussion

With this pilot study we aimed to explore if rTMS could influence the amplitude of CEOAEs. With the placebo-controlled Latin-square cross-over design we measured a small and significant increase in amplitude in all conditions, including the placebo condition. Therefore, with our results we could not determine if the efferent auditory pathway was stimulated by the rTMS and if it accounted for changes in OAE amplitude. We performed a small second experiment in which the use of earplugs on CEOAEs was demonstrated, showing a small increase of OAE-amplitude after wearing earplugs without any TMS intervention. Interestingly, the OAE-amplitude did not change after 15 minutes without intervention. This is in contrast to previously reported drifts in amplitude and frequency in spontaneous OAEs, when measured for 60 minutes (Whitehead, 1991).

The TMS clicks can reach a sound pressure level up to 121 dB at maximum stimulator level (Gilbert et al., 2004; Tringali et al., 2012). Recently, Tringali et al. (2012) tested hearing levels and TEOAE amplitude after real and sham TMS because of potential harmful side effects caused by the noise. All of their subjects wore disposable foam earplugs during the TMS. They did not find a change in hearing level. For TEOAEs they found a small, not significant increase in OAE amplitude, which is in line with our results. In subjects with the least protected hearing and the loudest TMS stimulation, they found a small decrease in OAE amplitude. They did not consider the earplugs or stimulation of the efferent auditory system as a potential source for the changes in OAE amplitude (Tringali et al., 2012). With our second experiment, we have demonstrated the increases in OAE amplitude after wearing earplugs in a small study group. Potentially, this effect could explain for the increase in amplitude found by Tringali et al. as well (2012).

The sensory innervation of the outer ear canal is performed mostly by the great auricular nerve and the auricular branch of the vagus nerve (e.g. Peuker and Filler, 2002). It is conceivable that an earplug can stimulate these nerves. To our knowledge no test has been performed to measure the influence of the prolonged stimulation of the great auricular nerve or the vagus nerve on OAE amplitude. But stimulation of the vagus nerve has been tested for its influence on the central auditory system. Invasive cervical vagus nerve stimulation (VNS) has been used to treat a variety of psychiatric and neurologic disorders, such as depression, epilepsy and Alzheimer's disease (Rush et al., 2000; Ben-Menachem, 2002). With electrical transcutaneous stimulation of the auricular

branch of the vagus nerve, Kraus et al. found decreased activity in the temporal and limbic regions of the brain when measured with fMRI (Kraus et al., 2007). Very recently, they confirmed their results with a sham controlled study design (Kraus et al., 2013). Whether the stimulation of the vagus nerve can also influence the cochlea and the OAE amplitude is for now highly speculative and remains to be tested. Based on our pilot study, OAE amplitude might increase with prolonged VNS due to wearing earplugs, making it interesting to explore in the future.

Occluding the ear with an earplug gives an elevated hearing threshold, but effects on the central auditory system are sparsely investigated. With an interesting study, Formby et al. (2003) demonstrated changes in (uncomfortable) loudness perception after peripheral auditory deprivation with earplugs. After wearing fitted earplugs almost continuously for 2 weeks, uncomfortable loudness levels decreased in all 8 subjects. This suggests that loudness perception is a plasticity phenomenon. One potential explanation for this effect was compensatory central gain in the central auditory system. This assumption was confirmed in a study with auditory reflex thresholds that were at a lower sound pressure level after wearing an earplug. Because effects were bilateral after unilateral plugging, the gain must be in the central auditory system (Munro and Blount, 2009). In our study, subjects wore earplugs for 15 minutes. It is demonstrated that within 10 hours of reduced auditory input, wave-I latency in brainstem electrical responses decreased in humans (Decker and Howe, 1981). Whether 15 minutes of wearing earplugs can give such an effect is not known.

Another potential explanation for the influence of earplugs on OAE amplitude is the temperature of the outer ear canal. It is feasible that prolonged occlusion of the outer ear canal increases its temperature with a small amount. To our knowledge, this has not been evaluated in humans. But increases in temperature could influence the OAE amplitude in 2 different ways. First, body temperature is reported to influence the middle ear compliance, in particular the stiffness of the tympanic membrane. Lowering the body temperature with 10° C in rats decreased the compliance of the middle ear, when measured with a tympanogram (Geal-Dor et al., 1997). They also found a modest linear correlation between the temperature and middle ear compliance (Geal-Dor et al., 1997). Increased middle ear compliance can also influence OAE measurements, to some degree comparable to the influence of middle ear muscle contractions (Guinan, 2006). A second explanation could be that hypothermia and hyperthermia are reported to influence OAE amplitude in humans (Ferber-Viart et al., 1995; Veuillet et al., 1997;

Seifert et al., 1998). Amplitude of OAEs generally decreased with relative large body temperature differences. The potential temperature differences in our study were not measured, but were probably too small to influence the cochlear amplifier itself. So although the results of our study seem to have been influenced by the wearing of earplugs, the exact mechanism remains speculative.

In the placebo condition in our study, the coil was tilted two-wing 90 degrees off the scalp. This prevents the electrical current to stimulate the cortex, but produces similar clicking noises as in the other conditions. It produces less scalp sensation than in actual stimulation (Loo et al., 2000), making it not an ideal placebo condition. This is especially true in a cross-over design study. The two-wing 90 degrees tilted placebo condition as used in this study, is tested to produce no motor-evoked potentials. It is devoid of any biological effects, as measured by Lisanby et al. (2001). Because CEOAEs are sounds that are generated unconsciously by the outer hair cells of the cochlea, we chose a placebo condition that can potentially be identified as placebo by the tested subject, but has the smallest chance to induce changes in the cortex excitability. Therefore, we feel confident that the placebo condition is adequate for our study setup.

There are some important limitations in this pilot study. A major limitation is the small number of subjects. The changes in OAE amplitude are generally so small, that a large group of subjects is needed to make a reliable statistical significant difference (Geven et al., 2012). Because we wanted to test the feasibility of influencing OAEs with TMS at first as a pilot study, we performed this with only a very small study population. This is especially true for the second experiment. Therefore, statistical results need to be assessed carefully. We also assumed that the potential influences of the rTMS would outlast the time it takes to measure the OAE signal. Although rTMS effects are reported to last longer than the actual stimulation, this is not known for the amplitude of OAEs. Potentially, the effect of rTMS on OAEs cannot be measured with this method due to the time it takes between stimulation and the measurement of the OAEs. In contrast, in the study by Perrot et al. the OAE measurements were within seconds after the cortical stimulation. Measuring OAEs during the stimulation seems not feasible due to the loud noise the figure-8 coil generates. Another limitation is the method of coil positioning. We did not use neuronavigation to position the TMS coil, so potentially we did not stimulate the primary auditory cortex with the small magnetic field. But recently, no difference was measured between neuronavigated rTMS and rTMS after coil positioning based on 10-20 EEG localization, as used in this study (Langguth et al., 2010; Langguth et al., 2012).

Conclusion

In conclusion, in this study we have tried to manipulate the efferent auditory system with indirect electrical stimulation of the primary auditory cortex in healthy subjects using rTMS. We were interested in the influence of rTMS on the OAE amplitude, but we were not able to demonstrate this, because OAE amplitude changes were also present in the placebo condition. Potentially, the earplugs worn by the subjects during the stimulation were responsible for this effect. With a small pilot study, we confirmed this suspicion, although data need to be reproduced in a larger study group. The cause for the change in OAE amplitude with earplugs remains speculative.

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Chapter 7

General discussion and conclusion

7.1 Introduction

Tinnitus is a bothersome phantom sound percept and its neural correlates are not yet disentangled. It is an increasing general health problem, with millions of people worldwide affected by it. With increasing age and increasing hearing loss in the general population, the incidence is thought to only rise in the near future, making tinnitus a highly relevant research topic. The scientific interest in tinnitus grows enormously, with an increasing number of publications every year. In 2002 a total of 176 tinnitus papers were published, and this number has increased to 405 papers in 2012 (Web of Science). Despite the increasing scientific interest, the exact nature of the origin of tinnitus is still not clear.

Tinnitus is associated with hearing loss and cochlear damage. Patients frequently report the perceived sound to be present in one or both ears. Therefore, a local cause within the cochlea was thought to cause tinnitus, although in the Babylonian Talmud it was already referred to as "buzzing of the brain". Cutting the vestibulocochlear nerve (for removal of acoustic tumours) led to tinnitus in 50% of the subjects that did not experience tinnitus before the surgery (Berliner et al., 1992). This contributed to the hypotheses that tinnitus is a central, rather than a peripheral auditory phenomenon.

The exact central mechanism that is responsible for causing tinnitus is unknown. Hypotheses on the pathophysiology of tinnitus generally concern neuroplastic changes in the central auditory system, probably initiated by some form of cochlear damage. As in every other efferent sensory system, the efferent auditory system, as a top-down control mechanism, is thought to participate in regulation and feedback of activity and inhibition in the central auditory system. Abnormal functioning of this system could therefore contribute to the plasticity involved in tinnitus, but this is currently mostly overlooked (Jastreboff, 1990; Bauer, 2004; Kaltenbach, 2011). Therefore, this thesis aimed to obtain more insight into the origin of tinnitus with special emphasis on the efferent auditory system.

In the following text the main findings and potential future implications will be discussed. Ideas for further research are presented.

7.2 Inner and outer hair cells and tinnitus

Despite the increasing number of published papers, the exact mechanism of the origin of tinnitus remains unclear. That some form of cochlear damage is related to tinnitus is evident, but why not all people with cochlear damage experience tinnitus is not known.

Recently, the role of the inner hair cells (IHCs) in the cochlea in tinnitus was investigated in detail (Knipper et al., 2013). Cochlear damage in the form of noise-induced hearing loss first results in loss of outer hair cells (OHCs) (Spoendlin, 1985). Total OHC loss accounts for about 40 dB of the total threshold loss. But noise-induced OHC loss can be accompanied by IHC loss as well (Liberman and Dodds, 1984a; Liberman and Dodds, 1984b). The loss of OHCs and IHCs alters or reduces the afferent input to the central auditory system. Research in mice showed that, after a mild noise trauma, partial deafferentation of IHCs occurred. There was only a temporary threshold shift, and there was recovery of OHC function, when measured by distortion product OAEs (Kujawa and Liberman, 2009). Such partial deafferentation is visible as a partial loss of synaptic ribbons of IHCs (Kujawa and Liberman, 2009). This loss of IHC ribbons has now been linked to tinnitus. Recently, it was demonstrated that IHC ribbon loss is related to behavioural evidence of tinnitus in rats (Ruttiger et al., 2013; Singer et al., 2013). These findings provide potential new insights into the molecular basis of tinnitus.

The results of Rüttiger et al. (2013) and Singer et al. (2013) indicate that tinnitus may be associated with the function of the ribbon synapses of IHCs. This suggests that the OHCs are not specifically involved in tinnitus. As OHCs are responsible for the generation of otoacoustic emissions (OAEs), it would be expected that emission characteristics are similar in subjects with and without tinnitus, respectively. In chapters 4 and 5 of this thesis we studied OAEs in tinnitus patients and specifically probed the function of the medial olivocochlear (MOC) efferent system, because of previously reported dysfunction of the MOC in tinnitus patients. The MOC system is the most peripheral portion of the efferent auditory system and can be tested non-invasively in humans. The MOC system suppresses OAEs by its efferent control of OHCs when stimulated with contralateral sound. In chapter 4 we concluded that contralateral suppression of OAEs for tinnitus patients was present and within normal ranges. Compared to a control group, suppression was equal, except for two out of five frequency bands (centered at 2.0 and 2.8 kHz) in tinnitus patients' right ears, where suppression was less pronounced. Apparently, the MOC is functional in suppressing the activity of the OHCs and basilar

membrane vibration in tinnitus patients. We could not explain the minor difference between tinnitus and control subjects, which could suggest a subtle difference between both study groups. Therefore, in chapter 5 we studied contralateral suppression with wavelet analysis, a method that includes both time and frequency information that is present in the original signal of the OAE. We showed that suppression of click-evoked OAEs in tinnitus patients was comparable with that in subjects without tinnitus in both time and frequency. Both our studies taken together give no indication for abnormal functioning of the MOC system in tinnitus patients. Thus, we found no evidence for dysfunction of the peripheral efferent system in tinnitus. The lack of abnormalities in the OAEs of tinnitus patients can be explained by the hypothesis that IHC ribbon loss rather than OHC dysfunction contributes to tinnitus.

In chapter 2 we suggested to test the reflex strength of the MOC as an additional method to see if a difference between tinnitus patients and controls could be demonstrated. A potential prospective study was proposed to test if the strength of the MOC reflex could help to predict individuals who are at risk to develop tinnitus after noise-induced cochlear damage. The limitation for such a study is that again only the brain stem level of the efferent auditory system and the OHC mediated effects are tested. If tinnitus is caused by IHC damage, MOC reflex strength will not be able to demonstrate differences between tinnitus patients and controls.

7.3 Tinnitus and hyperactivity

Functional imaging is an interesting avenue to study tinnitus patients. A potential ultimate goal is to try to objectify the currently *subjective* tinnitus. In an extensive review, the different imaging modalities with their advantages and disadvantages are discussed (Lanting et al., 2009). Unfortunately, the conclusions with fMRI and PET research are still mostly based on group data, and not on individuals. So trying to objectify the subjective phantom percept in an individual patient is still a future goal.

If tinnitus is related to increased neural activity in the (auditory) cortex, this might be detectable by positron emission tomography (PET) scans. PET scans are extensively used in e.g. oncological diagnostics to identify areas in the body with enlarged metabolic activity. In previously published research, tinnitus was shown to relate to increased metabolic activity in the left primary auditory cortex (Arnold et al., 1996; Langguth et

al., 2006). In chapter 3 we have confirmed that [^{18}F]-fluoro-deoxyglucose (FDG)-PET activity in the left PAC was higher than in the right PAC. However, this asymmetry was similar in tinnitus patients and control subjects. Therefore it cannot be the correlate of tinnitus. Remarkably, there was no evidence of areas with hyperactivity in the auditory cortex of tinnitus patients.

Instead of finding areas of hyperactivity, we found two areas with reduced activity in tinnitus patients. One of the areas is the left middle frontal gyrus (MFG). This area was also identified by Golm et al. (2013), who were able to correlate the measured brain activity with the reported tinnitus-related distress of the patients. Involvement of the limbic system in tinnitus has been widely discussed, because of the obvious stress and depressive symptoms some patients experience (for example Møller, 2006; Rauschecker et al., 2010; Golm et al., 2013). Potentially, the limbic system is responsible for the tinnitus burden, after the neuroplastic changes occurring with the cochlear damage. Careful interpretation of results is needed however, as most tinnitus patients included in research studies are from highly specialised tinnitus outpatient clinics. Therefore, we have set up a study to compare the tinnitus distress of patients from a tertiary referral center (University Medical Center Groningen) to a non-specialised secondary referral center (Isala Zwolle). If large differences are found in tinnitus distress between both groups, evaluating these groups with functional imaging with special emphasis on the limbic system is worthwhile. The aim would be to disentangle the actual tinnitus "sound" from the tinnitus "distress".

The results of our PET scan study in chapter 3 fit the hypothesis that tinnitus is not the result of increased baseline activity, but rather of increased synchrony in neural activity (Norena and Farley, 2013). With their extensive review, Noreña and Farley emphasize the important role of the homeostatic plasticity reaction in the central auditory system after the peripheral damage. They propose that tinnitus is a by-product in striving for balance in activity, with potential increased temporal coherence resulting in neural activity (Norena and Farley, 2013). They also suggest that functional coupling between various auditory structures may be important to consider in the changes in neural activity. Increased neural synchrony would presumably not be detected by FDG-PET, which might explain why we could not demonstrate hyperactive areas with FDG-PET.

7.4 Tinnitus and plasticity: a role for the efferent auditory system?

With this thesis we were not able to show a specific role of the efferent auditory system in the pathophysiology of tinnitus. In my personal opinion however, a system as large as the efferent auditory system with all its loops, chains and branches, which in general is involved in learning and modulation of feedback, and able to influence input at all levels within the auditory system, must be involved in some way to the reaction following the cochlear damage. Changes in the balance between excitation and inhibition are likely to play a role and the efferent auditory system can be expected to be involved in such changes. Unfortunately, we still do not know the exact nature or location of these changes. However, individual differences in the reaction of the efferent system to cochlear damage might explain why only a subgroup of the subjects with hearing loss experiences tinnitus. The efferent auditory system is possibly the factor that determines whether a patient's increased spontaneous neural activity after cochlear damage results in tinnitus. It will be worthwhile to investigate the efferent auditory system and its relation to tinnitus, but it is not easy. Due to the obvious limitations with research in humans, only "indirect" experiments can be thought of. In chapter 2, we gave some suggestions to start this line of research. Whether the efferent auditory system itself is responsible for an aberrant plastic reorganisation in response to cochlear damage, remains only speculative at this point.

To probe the efferent auditory system in humans we performed a pilot study. In chapter 6 we have tried to activate the efferent auditory system with indirect electrical stimulation of the primary auditory cortex in healthy subjects. The study was based on the published results of Perrot et al. (2006), who demonstrated the existence of a functional efferent auditory system in humans with direct electrical stimulation. In an elegant study, they stimulated the human auditory efferent system from cortex to cochlea by means of intra-cerebral electric stimulation of the auditory cortex. Stimulation of the auditory areas resulted in a significant reduction of the contralateral evoked OAE amplitude, whereas stimulation of the non-auditory areas showed no reduction in OAEs. Other research groups have not yet reproduced these data, but it would be very welcome.

With the study described in chapter 6 we aimed at activating the efferent system. In contrast to the direct electrical stimulus applied by Perrot et al. (2006), we applied

indirect transcranial magnetic stimulation (TMS). We were unable to demonstrate an effect of TMS on OAEs. However, OAEs did change during our experimental procedures. Additional experiments showed that the earplugs that were used during the experiments were possibly responsible for these changes. The effect of earplugs is possibly mediated by somatosensory excitation of the skin in the ear canal, which then feeds back to the cochlea, potentially via the efferent auditory system. Alternative explanations consider cochlear adaption to the change of acoustic impedance of the ear canal due to the earplugs. At present, we have no convincing explanation for the mechanisms responsible for the effect of earplugs. However, the interesting possibility of neural feedback mechanisms merit further study.

7.5 Future perspectives

With so many unanswered questions regarding the pathophysiology of tinnitus, it is not so easy to suggest the best next step to take in research.

As tinnitus seems to be the result from neuroplasticity or changes in homeostatic balance and neural activity, it will be interesting to see what happens to the tinnitus percept over the years in individual patients. Neuroplasticity is not constant during a lifetime, and may occur mostly during "sensitive periods of time" (Bischof, 2007). A prospective study to follow tinnitus patients for many years could provide insight into the perception on tinnitus and the potential changes during those years. Is the tinnitus loudness something patients can adapt to? Are there some patients where the tinnitus completely resolves in time? Can the auditory system adapt to a new balance? Unfortunately, repeated psychoacoustic measurements of tinnitus loudness or pitch are somewhat unreliable for a prolonged period of time (Henry and Meikle, 2000). Self-reported tinnitus loudness and annoyance were reported to have a test-retest reliability of 0.72 and 0.62 respectively (Zenner and De Maddalena, 2005). The Tinnitus Handicap Inventory questionnaire (THI, Newman et al., 1996) was reported to be robust in internal consistency, it was sensitive to tinnitus change and it was able to differentiate between patients with different tinnitus severities (Meikle et al., 2007; Zeman et al., 2012). So, long-term follow-up with self-reported tinnitus severity and annoyance, and the THI seems reliable and is easy to execute. This would make a good start for a longitudinal study in tinnitus patients.

Another interesting way to evaluate the potential changing plasticity in tinnitus is to evaluate tinnitus in children. The prevalence of tinnitus in children is estimated to be 6 to 36%, depending on whether children reported it spontaneously or whether they were asked about it (reviewed by Shetye and Kennedy, 2010). This number is even higher in hearing impaired children (Savastano et al., 2009; Juul et al., 2012). In adolescents, the prevalence of tinnitus is estimated at 31 to 37% (Bulbul et al., 2009). It is not known if the children who report tinnitus, will still perceive the sound when reaching adult life. It is well known that the potential for neural plasticity is significantly greater in young, still developing brains as compared to adult brains (for example see Bischof, 2007). Because tinnitus is believed to be related to plasticity effects after cochlear damage, the enhanced plasticity of childrens' brains can be of special interest. Therefore, tinnitus would need to be asked regularly in children with hearing impairment during their follow-up at the otorhinolaryngology clinic. Longitudinal changes in the prevalence and incidence could give an insight in the role of plasticity in children with tinnitus.

A third great opportunity to examine plasticity effects in the auditory system is when deaf patients receive a cochlear implant. A great number of patients report tinnitus prior to implantation; after cochlear implantation both suppression and increase of tinnitus are reported (reviewed by Quaranta et al., 2004; Arts et al., 2012). Cochlear implantation restores peripheral stimulation, thereby potentially rewinding the neuroplastic changes that have occurred with the deterioration of the hearing level. It would be interesting to see if brain metabolism changes after receiving a cochlear implant. A major limitation is that fMRI cannot be used to test these functional changes, due to artefacts rising from the implant. FDG-PET scanning on the other hand is usable, but has a poorer resolution.

Another potential device that could restore or influence auditory input is an auditory brainstem implant (ABI). This device restores hearing to some degree with electrical, tonotopic stimulation of the cochlear nucleus. This device is used when cochlear implantation is not feasible, for example in patients with neurofibromatosis type 2 (Colletti et al., 2012). Potentially, the direct influence on the cochlea nucleus with the electrical stimulation of the ABI changes tinnitus percept. Although only limited numbers of patients are suitable to receive an ABI, it would be of interest to investigate the effect on the tinnitus perception (Soussi and Otto, 1994).

Conclusion

This thesis describes some experiments that aimed to obtain more insight into the origin of tinnitus with special emphasis on the efferent auditory system. With different techniques, various parts of the central auditory system have been investigated. We have demonstrated that tinnitus is not related to hyperactivity in the left primary auditory cortex. Treatment protocols targeted at reducing this supposed hyperactivity lose their rationale with this finding.

We did not demonstrate dysfunction of the efferent system in tinnitus at the level of the lower brain stem. But the rest of the efferent system remains open for investigation. Several potential avenues for research are suggested in this thesis. Whether the efferent auditory system plays a specific role in tinnitus remains speculative at this point.

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Chapter 8

Summary & Samenvatting

Summary

Tinnitus is a percept of a meaningless sound without an external source. The word tinnitus comes from the Latin word "tinnire", which means to ring. In fact, tinnitus is often referred to as "ringing in the ears". Transient tinnitus is experienced by almost all adults at some point in their life. Tinnitus can also be permanent and up to 1-3% of the people with tinnitus are severely affected by this and seek medical attention. This bothersome tinnitus can only be heard by the patient and not by others. Because there is no identifiable sound source, tinnitus is thought to be a phantom percept. The pathophysiology of tinnitus is not known, and therefore there is increasing scientific interest. Tinnitus is currently considered to involve central phenomenon in the brain including auditory areas, although some form of cochlear damage probably initiates the neuroplastic changes in the brain, that results in tinnitus.

This thesis concerns the pathophysiology of tinnitus, with special emphasis on the efferent part of the central auditory system. The efferent auditory system runs from the auditory cortex to the cochlea, connecting all auditory regions along its path. As in every other efferent sensory system, the efferent auditory system is thought to participate in regulation and feedback of activity. Abnormal functioning of this system could therefore contribute to the plasticity involved in tinnitus.

Chapter 1 contains the general introduction to tinnitus and the central afferent and efferent auditory system. In addition, the aim and the outline of the thesis are stated. The aim of the thesis is to obtain more insight into the origin of tinnitus with special emphasis on the efferent auditory system.

In **chapter 2** we investigated the current knowledge of the functional efferent auditory system in humans. We performed a literature review, mostly based on animal research. We looked for new possibilities in trying to understand the specific role of the corticofugal efferent auditory system in tinnitus. We made several suggestions for future research, for studies in humans as well as in animals to investigate the efferent auditory system and its relation to tinnitus.

In **chapter 3** we studied the auditory cortex in tinnitus with [18F]-fluoro-deoxyglucose positron emission tomography (FDG-PET). Previously published papers using FDG-PET have suggested an increased metabolism in the left primary auditory cortex in tinnitus patients. The purpose of this study was to test whether left-sided hyperactivity in the

auditory cortex is specific to tinnitus or is a general characteristic of the auditory system unrelated to tinnitus. Therefore, FDG-PET was used to measure brain metabolism in 20 tinnitus patients. Their results were compared to 19 control subjects without tinnitus. The activity in the left primary auditory cortex was higher than in the right primary auditory cortex, but this asymmetry was present in both tinnitus patients and control subjects. In contrast, the lateralization in secondary auditory cortex was opposite, with higher activation in the right hemisphere. These data showed that hemisphere asymmetries in the metabolic resting activity of the auditory cortex are present, but these are not associated with tinnitus and are a normal characteristic of the normal brain. In contrast to our expectation, there was no hyperactivity associated with tinnitus.

A small part of the human efferent auditory system can be tested non-invasively with otoacoustic emissions (OAEs) and their response to contralateral acoustic stimulation. Stimulation of the medial olivocochlear efferent system is responsible for this reduction of OAEs after contralateral acoustic stimulation. In **chapter 4** we compared the functioning of the medial olivocochlear efferent system between 97 tinnitus patients and 44 control subjects. We used suppression of click-evoked OAEs with contralateral acoustic stimulation to test the hypothesis. Suppression was calculated in half-octave frequency bands centered at 1.0, 1.4, 2.0, 2.8, and 4.0 kHz. We found that OAE amplitudes and contralateral suppression were equal in both groups. The amount of suppression was equal, except for the 2.0- and 2.8-kHz frequency bands in the right ear, for which the patients had less suppression. The minor differences between both groups could have suggested subtle differences in the function of the medial olivocochlear efferent system in tinnitus patients.

Since we found minor differences in contralateral suppression between tinnitus patients and controls, we analyzed the outcome more extensively in **chapter 5.** With wavelet analysis both time and frequency information of an emission can be analyzed and compared. Contralateral suppression of OAEs was therefore analyzed using wavelets. No significant difference in suppression was found between the tinnitus patients and the control group. Therefore, we found no indication for abnormal functioning of the medial olivocochlear system in tinnitus patients.

To investigate the efferent system in humans from cortex to cochlea, we designed a study described in **chapter 6.** The functionality of the human efferent auditory system from cortex to cochlea has been demonstrated with –invasive- electrical stimulation. Stimulation of the auditory cortex resulted in decreased contralateral OAE amplitude.

An alternative way to stimulate brain regions is by transcranial magnetic stimulation (TMS). We explored the effect of TMS of the auditory cortex on contralateral OAEs to test for efferent auditory system function. We compared OAE amplitude directly before and after TMS. The subjects wore earplugs during TMS stimulation, to protect against the acoustic pulses produced by the TMS equipment. Because we found increases in OAE amplitudes in all TMS conditions, including the placebo condition, an additional control experiment was conducted, in which subjects also wore earplugs for 15 minutes without intervention. After passively wearing earplugs for 15 minutes, the OAE amplitude also increased significantly. Passive waiting without earplugs did not change the OAE amplitudes. With this study we were not able to demonstrate that an increase in OAE amplitudes was related to TMS of the auditory cortex. With a small pilot study, we demonstrated similar increases in OAE amplitude after wearing earplugs without stimulation. The cause for the change in the amplitude with earplugs remains speculative.

In **chapter 7** an overview of the thesis with its relation to the published literature is given. The role of recently published studies concerning inner hair cell damage in tinnitus is discussed, with its relations to the results described in chapter 4 and 5. The relation between tinnitus, functional imaging and the limbic system is also briefly discussed.
Building on the special emphasis of this thesis on the efferent auditory system, potential new avenues for other research projects are discussed. With the large difference in emotional distress between patients, a new research project to compare tertiary referral patients with secondary referral patients is explained. The potential value of long-term follow-up of tinnitus patients to explore the role of neuroplasticity is pointed out. Especially in children, in whom tinnitus is seldom reported, the influence of neuroplasticity will be interesting. Patients with severe sensorineural deafness who are treated with a cochlear implant or an auditory brainstem implant are other examples for neuroplasticity research in tinnitus.

In conclusion, this thesis describes some experiments that aimed to provide more insight in the pathophysiology of tinnitus, with special emphasis on the efferent auditory system. We have demonstrated that asymmetry in metabolism in the primary auditory cortex previously associated with tinnitus is a normal characteristic of the human brain. We did not detect areas of hyperactivity in the cortex with FDG-PET scanning. We also did not detect abnormalities in the medial olivocochlear efferent system in tinnitus patients. Several suggestions for future research are discussed in the various chapters.

Samenvatting

Tinnitus, oftewel oorsuizen, is het waarnemen van een geluid, waar geen externe bron voor is. Tinnitus kan alleen worden waargenomen door de persoon zelf en niet door anderen. Omdat er geen aanwijsbare bron van het geluid is, wordt ervan uitgegaan dat tinnitus een "fantoomgeluid" is. De meeste volwassenen hebben wel eens last gehad van tijdelijke tinnitus, soms na blootstelling aan hard geluid. Tinnitus kan echter ook blijvend zijn. Geschat wordt dat permanente tinnitus bij 8-20% van de mensen in de algemene bevolking voorkomt, waarbij de meeste mensen goed met deze klacht kunnen omgaan. Echter, 1-3% van de mensen met tinnitus heeft zoveel klachten dat ze medische hulp zoeken. Deze patiënten hebben zoveel last dat de tinnitus zorgt voor een meetbare vermindering in kwaliteit van leven.
De oorzaak van tinnitus is nog niet precies bekend. Momenteel gaat men ervan uit dat bij tinnitus veranderingen in de centrale hersengebieden, inclusief de hersengebieden die betrokken zijn bij het horen (het centrale auditieve systeem), een bepalende rol spelen bij het ontstaan van tinnitus. Het ontstaan wordt waarschijnlijk vooraf gegaan door een vorm van schade aan het gehoor of gehoororgaan. Deze schade zorgt dan voor de veranderingen in het brein.

Dit proefschrift behandelt de pathofysiologie van tinnitus en in het bijzonder de rol van het efferente deel van het centrale auditieve systeem. Het efferente auditieve systeem loopt van de hersenschors (de auditieve cortex) naar het slakkenhuis (de cochlea) en verbindt daarmee alle auditieve gebieden met elkaar. Het meer bekende afferente auditieve systeem verloopt in tegengestelde richting, van de cochlea naar de auditieve cortex. Het efferente auditieve systeem speelt vermoedelijk een rol in de regulatie en feedback van activiteit, zoals alle efferente sensorische systemen doen. Hierdoor zouden afwijkingen in dit systeem kunnen zorgen voor de veranderingen in het brein, die bij tinnitus een rol spelen.

In **Hoofdstuk 1** wordt een samenvatting gegeven van de huidige wetenschappelijke literatuur over tinnitus. Het centrale auditieve systeem wordt besproken, zowel het afferente als het efferente deel. Ook wordt het doel van dit proefschrift uitgelegd, namelijk om meer inzicht te krijgen in het ontstaan van tinnitus, waarbij speciaal gekeken is naar het efferente systeem.

In **Hoofdstuk 2** is de huidige kennis over het functionele efferente auditieve systeem verzameld. Dit betreft voornamelijk onderzoek in proefdieren, maar waar mogelijk ook gegevens over onderzoek in mensen. Met deze literatuurstudie hebben we gezocht naar mogelijkheden om de rol van het efferente auditieve systeem in tinnitus te onderzoeken. We geven meerdere suggesties voor onderzoeksmogelijkheden in zowel mensen als proefdieren. Deze onderzoeken zouden als doel hebben om de rol van het efferente auditieve systeem in het ontstaan van tinnitus beter te begrijpen.

Hoofdstuk 3 beschrijft ons onderzoek naar de auditieve cortex. Met een beeldvormende techniek ([18F]-fluoro-deoxyglucose positron emissie tomografie (FDG-PET)) is in eerdere studies beschreven dat het basale metabolisme van de linker auditieve cortex in tinnituspatiënten hoger is dan in de rechter. Het doel van onze studie was om te onderzoeken of deze linkszijdige verhoogde activiteit veroorzaakt werd door tinnitus, of dat dit een kenmerk van het normale auditieve systeem was. Daarom hebben we de FDG-PETscans van 20 tinnituspatiënten vergeleken met die van 19 gezonde proefpersonen. De resultaten toonden inderdaad een verhoging van activiteit in de primaire auditieve cortex links, dit was echter aanwezig in zowel de patiënten als de controles. Voor de secundaire en associatieve auditieve cortex was de activiteit juist hoger aan de rechterzijde, zowel voor de patiënten als de controles. Blijkbaar is asymmetrie in het basale metabolisme van het centrale auditieve systeem aanwezig, maar dit is geen kenmerk dat wordt veroorzaakt door tinnitus. Dit heeft consequenties voor de tinnitustherapieën die zijn ontwikkeld met als doel de vermeende verhoogde activiteit van de linker auditieve cortex te verlagen. In tegenstelling tot onze verwachting vonden we met dit onderzoek geen andere hersengebieden met verhoogde activiteit in de tinnituspatiëntengroep.

Een klein gedeelte van het efferente auditieve systeem kan in mensen onderzocht worden zonder invasieve technieken. Dit deel is het mediale olivocochleaire (MOC) efferente systeem, en bevindt zich op hersenstamniveau. Dit kan onderzocht worden door otoakoestische emissies (OAE's) te meten. Dit zijn geluiden die worden gegenereerd in de cochlea en kunnen worden gemeten in de gehoorgang. Deze OAE's kunnen worden beïnvloed door geluid aan te bieden aan het andere oor. Door het aangeboden geluid wordt het MOC systeem geactiveerd, waardoor via de efferente verbindingen de haarcellen in de contralaterale cochlea worden beïnvloed. Dit is meetbaar met behulp van de OAE's. In **Hoofdstuk 4** hebben we het functioneren van het MOC systeem onderzocht in 97 tinnituspatiënten. De reactie van de OAE's op

contralateraal geluid is vergeleken met de resultaten van 44 controledeelnemers. De mate van reductie van de OAE's, genaamd suppressie, is als uitkomstmaat gebruikt. Onze resultaten toonden dat de hoogte van de OAE's in beide groepen gelijk waren. De suppressie was hetzelfde voor de meeste frequentiebanden, behalve rond de 2,0 en 2,8 kHz in het rechteroor. Voor deze frequenties was de suppressie in de tinnituspatiënten lager dan in de controledeelnemers. Dit kleine verschil zou kunnen duiden op een subtiel verschil in het functioneren van het MOC systeem in tinnituspatiënten.

Vanwege de kleine verschillen in de suppressie van de OAE's tussen tinnituspatiënten en de gezonde controledeelnemers, hebben we een aanvullende analyse uitgevoerd. Dit staat beschreven in **Hoofdstuk 5**. We hebben een techniek genaamd *wavelet analysis* gebruikt om de suppressie van de tinnituspatiënten met de controles te vergelijken. Bij *wavelet analysis* wordt niet alleen de frequentie-informatie gebruikt, maar ook de tijdsinformatie die aanwezig is in het signaal. Met *wavelet analysis* vonden we geen significante verschillen tussen de tinnituspatiënten en de controles. We hebben dus geen aanwijzingen dat het MOC-systeem in tinnituspatiënten anders functioneert dan in gezonde controles.

Om het gehele menselijke efferente auditieve systeem, van cortex naar cochlea, te onderzoeken, hebben we een studie ontworpen. Deze studie wordt beschreven in **Hoofdstuk 6**. De functionaliteit van het humane efferente auditieve systeem van de auditieve cortex naar de cochlea is aangetoond met –invasieve– elektrische stimulatie van de auditieve cortex. Deze stimulatie van de auditieve cortex leidde tot suppressie van de OAE-amplitude in het contralaterale oor. De cortex kan ook op een andere manier gestimuleerd worden, namelijk met behulp van transcraniële magnetische stimulatie (TMS). TMS stimuleert heel lokaal corticale hersengebieden door een snel wisselend magnetisch veld. Deze techniek is niet invasief. Wij hebben het effect van TMS op de contralaterale OAE's getest, om op deze manier het gehele functionele efferente auditieve systeem te onderzoeken. We hebben de OAE-amplitude direct voor en na TMS vergeleken bij gezonde proefpersonen. Tijdens de TMS droegen de deelnemers oordoppen om ze te beschermen tegen het harde geluid van de TMS-apparatuur. In alle condities, dus ook de placebo-conditie, vonden we een verandering van de emissies. Daarom is aanvullend een tweede experiment uitgevoerd waarbij de deelnemers oordoppen hebben gedragen gedurende 15 minuten zonder andere interventie. Na het dragen van de oordoppen waren de emissies significant veranderd. Na 15 minuten wachten zónder oordoppen, veranderde de OAE-amplitude niet. Met

deze studie hebben we dus helaas niet het effect van TMS op de amplitude van de OAE's kunnen bestuderen. Wel hebben we, in een kleine groep, een effect van het dragen van oordoppen op de emissies aangetoond. De verklaring voor de verandering in de emissies door het dragen van de oordoppen is vooralsnog speculatief.

In **Hoofdstuk 7** worden alle resultaten van dit proefschrift in relatie gebracht met de huidige wetenschappelijke literatuur over tinnitus. Vanuit de speciale aandacht voor het efferente auditieve systeem in dit proefschrift, doen wij enkele suggesties voor nieuwe studies. Vanwege het mogelijke grote verschil in emotionele beleving en last tussen academische en perifere tinnituspatiënten, wordt een nieuw onderzoek uitgelegd. Voor dit onderzoek zal de emotionele last die veroorzaakt wordt door de tinnitus vergeleken worden tussen patiënten van het Isala ziekenhuis in Zwolle en het Universitair Medisch Centrum Groningen.
De waarde van het langdurig vervolgen van tinnituspatiënten wordt uitgelegd. Met het langdurig vervolgen van patiënten willen wij de rol van neuroplasticiteit bij het ontstaan van tinnitus bestuderen. In het bijzonder in kinderen, waar tinnitus waarschijnlijk (te) weinig wordt gerapporteerd, is de rol van neuroplasticiteit interessant om te onderzoeken. Ook patiënten met zeer ernstige perceptieve slechthorendheid en tinnitus, die behandeld zullen worden met een cochleair implantaat of een *auditory brainstem implant,* zijn interessant om te vervolgen om een indruk te krijgen van de rol van neuroplasticiteit in tinnitus.

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Curriculum Vitae

Leontien Ingeborg Geven werd, als oudste van drie dochters, geboren op 29 juni 1982 te Nijmegen. Haar jeugd heeft ze tot haar 15de doorgebracht in Nijmegen en omstreken. Daarna is zij verhuisd naar Groningen, waar zij in 2000 haar eindexamen haalde op het Praedinius Gymnasium. Direct aansluitend kon zij beginnen met de studie Geneeskunde aan de Rijksuniversiteit Groningen. Tijdens haar studie werkte ze onder andere als onderzoeker mee aan het POPS-19 onderzoek, een onderzoek naar de lange termijngevolgen bij 19-jarige ex-prematuren. Haar wetenschappelijke stage onder enthousiaste en inspirerende leiding van professor T. Ebels resulteerde in de publicatie getiteld "Vascular thoracic outlet syndrome. Longer posterior rib stump causes poor outcome". Haar co-schappen vervulde zij in het Deventer Ziekenhuis, alwaar haar interesse voor de KNO-heelkunde werd gewekt. Hier heeft zij de unieke mogelijkheid gekregen om onder leiding van drs. H. Jensma, KNO-arts in ruste, mee te mogen op één van de missies van stichting "Eardrop", naar Eldoret in Kenia. Haar afsluitende keuzeco-schap werd verricht op de KNO-afdeling van het UMC St Radboud te Nijmegen. Gedurende deze tijd heeft zij onder begeleiding van professor K. Graamans en dr. J.J. Mulder een case-report gepubliceerd, getiteld "Giant cholesteatoma: recommendations for follow-up". Haar artsenbul ontving in september 2006.

In november 2006 is zij begonnen met werken op de KNO-afdeling van het UMCG. De eerste 6 maanden als ANIOS, maar daarna in een traject dat dit promotieonderzoek combineerde met de opleiding tot KNO-arts. Haar perifere stages deed zij in het Martini Ziekenhuis Groningen en de Isala Klinieken te Zwolle. Zij verwacht haar opleiding tot KNO-arts succesvol af te ronden op 1 mei 2014.

Dit seizoen is ze voor het vijfde jaar betrokken bij het skûtsjesilen in de Iepen Fryske Kampioenskippen Skûtsjesilen (IFKS). Met het wedstrijdskûtsje "de Yde" zal ze als stuurboord zwaardvrouw samen met de rest van de bemanning proberen dit jaar uit de C-klasse van de IFKS te promoveren.